COMPARATIVE EVALUATION OF THE EFFECT OF DIFFERENT REMINERALIZING AGENTS ON ARTIFICIAL CARIES LIKE ENAMEL LESIONS USING SCANNING ELECTRON MICROSCOPE, SURFACE ROUGHNESS AND SURFACE MICRO HARDNESS ASSESSMENT-AN IN-VITRO STUDY

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

In partial fulfillment for the degree of

MASTER OF DENTAL SURGERY

BRANCH – VIII

PEDODONTICS AND PREVENTIVE DENTISTRY

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600032

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Dr. I.K.Ramyalakshmi

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LIST OF ABBREVIATIONS

S.No.	ABBREVIATIONS	EXPANSIONS
1	SMH	Surface Microhardness
2	R_a	Surface Roughness
3	SEM	Scanning Electron Microscope
4	CaSP	Calcium Sucrose Phosphate
5	HA_p	Hydroxyapatite
6	CPP-ACPF	Caesin Phosphopeptide – Amorphous Calcium Phosphate with Fluoride
7	WSLs	White Spot Lesions

ABSTRACT

BACKGROUND & OBJECTIVES

Dental caries, etiologically is a multifactorial disease because of both bacterial acid production along with the buffering action from saliva and the surrounding surface of the tooth structure earlier visible as White Spot Lesions (WSLs).

Remineralzation is the process whereby calcium ions and phosphate ions are supplied from an external source to the tooth to promote ion deposition into crystal voids. This study was done among various remineralizing agents containing different levels of calcium-phosphorous and fluoride delivery systems on incipient carious lesions and then analysed using Scanning Electron Microscope, Surface Micro Hardness analysis and Surface Roughness Analysis.

The objective is to compare and evaluate the remineralizing efficacy of various calcium-phosphate and fluoride delivery vehicles on artificial caries using Scanning Electron Microscope, Surface Micro Hardness analysis and Surface Roughness Analysis.

METHODS

Ninety two extracted human mandibular molars were and acid resistant varnish was then applied around the exposed enamel surface after leaving four equal windows and then immersed in demineralizing solution followed by remineralization under Artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix for duration of 4 week.

RESULTS

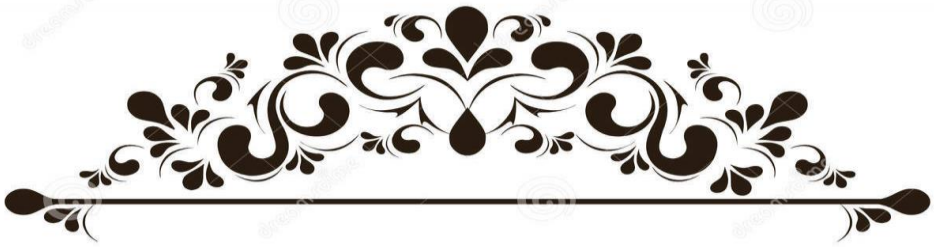
All the specimens on evaluation in SEM showed evidence of thickening of their inter-rod substance early by 4 week of remineralization and marked evidence of remineralization was evident in Tooth Mousse Plus. In final acid exposure, Enafix showed more resistance to dissolution. Surface Micro Hardness (SMH) and the Surface Roughness ($R_{a)}$ also exhibited similar results with marked reduction in roughness values and increased microhardness values with Tooth Mousse Plus exhibiting greater source of remineralization and Enafix showing more resistance to final acid challenge.

INTERPRETATION & CONCLUSION

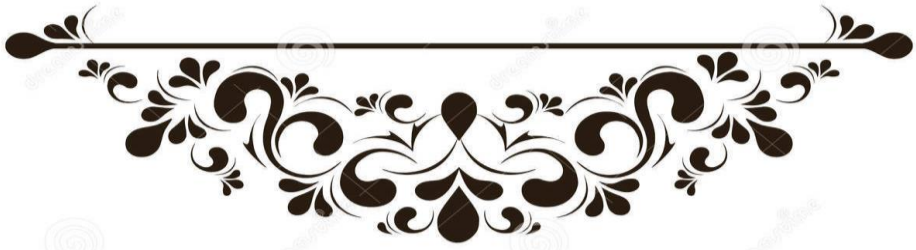
Tooth Mousse Plus has shown better remineralization properties while Enafix has shown better resistance to final acid challenge.

KEYWORDS

SMH – Surface Micro Hardness; R_a – Surface Roughness; SEM – Scanning Electron Microscope;



INTRODUCTION



INTRODUCTION

It is widely accepted that the oral and general health has greater impact in influencing the quality of life. According to the World Oral Health Report of 2003, oral diseases impede activities in school and work causing many productive hours to be lost each year, and this criteria exists all over the world. Despite all the worldwide improvements in the oral health, dental caries is still one of the major oral health problem in most industrialized countries that affects about 60% - 90% of school children and also the vast majority of adults too. Dental caries belongs to a group of multiple complex diseases and it ensues because of multiple contributing factors. Many strategies are nowadays being applied for the prevention of dental caries but not even a single strategy can guarantee 100% success.[1]

Dental caries etiologically is a multifactorial disease that results from complex interactions among tooth structure, dental biofilm, dietary and as well as salivary influences. This is a result of both bacterial acid production along with the buffering action from saliva and the surrounding tooth structure. The tooth surface is therefore in a dynamic equilibrium with its surrounding environment playing a vital role. Dental caries of the enamel is first observed clinically as a so-called ‘‘white spot lesion’’ which is a small area of sub-surface demineralization, beneath the dental plaque. The lesion appears white because the loss of mineral changes the refractive index compared with that of the surrounding translucent enamel. The body of the sub-surface lesion may have lost as much as 50% of its original mineral and often has an ‘‘apparently intact surface layer’’ over it.[2] The process of demineralization continues each time when carbohydrate is taken into the mouth and metabolized by the bacteria. The saliva plays numerous roles

including buffering (neutralizing) the acid and providing minerals that can replace those dissolved from the tooth during a demineralization challenge.

This replacement of mineral is called remineralization. The term remineralzation is so been defined as the process whereby calcium and phosphate ions are so been supplied from a source external to the tooth surface in order to promote ion deposition into the crystal voids of demineralized enamel to produce net mineral gain.[3]

As the pH falls below a critical value, the demineralisation of enamel, dentine or cementum occurs, while a gain of mineral (remineralisation) occurs as the pH increases. And so dental caries is a chronic, dietomicrobial, site specific disease caused by shifts from tooth protective factors favouring tooth remineralization to destructive factors leading to demineralization process. The process of demineralization and reminer-alization takes place more frequently during the day. Over time, this process leads to either caries lesions or the repair and reversal of a lesion. [3]

The current concept regarding cariogenesis is that a caries lesion, either clinically invisible or detectable and that results in accumulation of numerous episodes of demineralization as well as remineralization, rather than a unidirectional process of demineralization. The periods during which there is return to the resting pH is when the actual remineralization occurs. Thus, it can be stated that remineralization is the process by which partly-dissolved crystals are induced to grow by the accretion of calcium along with phosphate ions from the solution. Remineralization is an important natural repair process that counteracts cariogenic challenge.[1,3]

The ultimate goal of modern dentistry is so been to manage non-cavitated carious lesions in a non-invasive manner in an attempt to prevent further disease progression and preserve integrity of healthy tooth substrate. Non-invasive management of noncavitated carious lesions comprises of several strategies; among these is healing the demineralized

lesions using remineralizing agent is the common way of managing non-cavitated lesions. Remineralization is the process for noncavitated lesions relies on much of the calcium and phosphate ions assisted with fluoride to rebuild a new surface on the already existing crystal remnants in subsurface lesions after remaining demineralization. These remineralized crystals are less acid soluble than the original mineral.[4]

Zero et al 2006 proposed the requirements of an ideal remineralizing agent

- Diffuses into the subsurface or else they should delivers calcium and phosphate into the subsurface.
- It should not deliver an excess of calcium.
- Does not encourage calculus formation.
- It can work at an acidic pH.
- Works in xerostomic patients.
- Boosts the remineralizing properties of saliva. [5]

Fluoride is one of the most recognized remineralizing agent that interacts with oral fluids on the interface of enamel along with subsurface regions of teeth, and then combining with calcium and phosphate ions in order to form fluorapatite. The anticaries benefits of fluoride depends wholly upon the use of an effective concentration and frequency of application. Fluorides plays a key role in the prevention as well as the control of dental caries. There is no doubt that the discovery of the anti-cariogenic properties of fluoride was one of the most important landmarks in the history of dentistry.[6] Moreover Remineralization of incipient caries is accelerated by trace amount of fluoride. Reasons to find alternative to fluoride:

- The efficiency of fluoride is mainly for the caries in smooth surface.
- High fluoride exposures should be restricted in order to avoid fluorosis.

• In certain countries, fluoride exposures has been made limited.

• When used properly fluoride is found to be safe in normal individual but among certain conditions the fluoride exposures should be always limited as the toxicity of fluoride increases with nutritional deficiency (inadequate vitamin-mineral) and also in immune-compromised cases such as diabetes and renal disease.

• The strong antifluoride lobby is claiming that diseases like mental retardation, GI diseases, intestinal disorders, Down's syndrome, thyroid problems are mainly attributed to the over exposure of fluoride. [7]

Natural tooth substance consists largely of hydroxyapatite that chiefly consists up of calcium and phosphate ions. And the hydroxyapatite in the natural tooth substance is being mimicked in the commercially available product **REMIN PRO (VOCO)** that tends to fill the superficial enamel lesions as well as the tiniest irregularities that arises from demineralization and erosion. Remin Pro adheres to the tooth substance and protects the tooth against demineralization as well as erosion. The surface is noticeably smoothed and dentin tubules are superficially sealed. Furthermore, the smooth surface impairs the adhesion of the bacterial plaque on to it. On the tooth surface, fluoride is converted into the more stable and more acidresistant fluorapatite through contact with saliva. The fluoride (1,450 ppm fluoride) contained in Remin Pro strengthens the tooth suface and thus makes it more resistant to acid attacks. The sugar substitute Xylitol is known for its potential cariostatic properties. The important aspect of xylitol is that, (unlike saccharin, for example) they cannot be converted into harmful lactic acid by cariogenic bacteria. Thus the harmful effects of these bacteria and the metabolic products such as lactic acid can be significantly reduced by the action of Xylitol, allowing the mouth to naturally remineralize damaged teeth with lesser interruption. [8]

To now highlight the basic mechanism of dental caries provided by **Miller (1898)** and still accepted today is that dietary carbohydrate is taken up by the bacterial layer on the tooth surface (dental plaque) and metabolized, producing organic acid which attacks the enamel surface and removes the calcium phosphate mineral of which it is largely composed **Levine et al 1977.**[6]

And to overcome the above mentioned tooth surface loss a milk protein namely Casein Phosphopeptide-Amorphous Calcium Phosphate (CPP-ACP) was so been introduced as a remineralizing agent in the year of 1998. It comprises up of several nanocomplexes of milk protein that is CPP along with ACP. It has been claimed that they promote the remineralization process of the carious lesions by maintaining a supersaturated state of essential minerals, at the same time they also hinders colonization of dental surfaces by the cariogenic bacteria. CPP–ACP is a milk product that helps in remineralization and prevents dental caries. Casein phosphopeptide can deliver amorphous calcium phosphate and can also help the ACP to bind with the tooth structure. Casein phosphopeptide can also significantly decrease the count of Strept. Mutans as it has got the ability to integrate into the pellicle. CPP is a peptide which contains elements that can bind the calcium ion. Casein phosphopeptide can stabilize the calcium phosphate present in the solution as amorphous calcium phosphate. Several in vitro studies have shown the role of CPP–ACP in the reversal of the early white spot lesion in their studies.[8] Owing to the overwhelming role of fluoride in the remineralization process, it was added to most of calciumphosphate delivery products, in different forms, and concentrations. The remineralization efficacy of fluoride containing materials is dependent upon the fluoride content, fluoride matrices, setting mechanisms and other materials components.[9]

Longbottom C. et al 2009 proposed that an ideal caries preventive material must release calcium and phosphate in the oral environment. Therefore, manufacturers of novel caries preventive dental materials are now incorporating CPP–ACP in the composition of their products for preventing caries This technology was developed by Eric Reynolds and co-workers at the University of Melbourne, and has since been incorporated into chewing gums and tooth cream (**GC TOOTH MOUSSE PLUS™**) a formulation with incorporated fluoride to a level of 900 ppm (GC ToothMousse Plus ™). This protein nanotechnology combines the specific phosphoproteins from bovine milk with forming the nanoparticles of amorphous calcium phosphate (ACP). The precise ratio is about 144 calcium ions plus 96 phosphate ions and 6 peptides of CPP.[4]

The casein phosphopeptides (CPP) are so been produced from a tryptic digest of the milk protein called casein, then aggregated with calcium as well as phosphate and then purified by ultrafiltration method. Under specific alkaline conditions the calcium phosphate ions that are present as an alkaline amorphous phase are so been complexed by that of the CPP. The nano-complexes form over a pH about a range from 5.0 to 9.0. Under neutral and alkaline conditions, the casein phosphopeptides stabilize calcium and phosphate ions, forming metastable solutions that are supersaturated with respect to the basic calcium phosphate phases in the precipitate form. The amount of calcium and phosphate ions are bound by CPP that increases as pH rises, reaching the point where the CPP have bound their equivalent weights of calcium and phosphate to the enamel rods, and reform the apatite crystals CPP is able to stabilize amorphous calcium fluoride phosphate (CPP-ACFP) **TOOTH MOUSSE PLUS (GC INDIA)**, which allows additive effects on remineralization compared with the fluoride or CPP-ACP alone). Moreover, CPP-ACP promotes the incorporation of fluoride into plaque and sub-surface enamel,

producing effects superior to those which can be achieved using fluoride alone (Reynolds et al. 2006).[10]

And the newer mode of remineralization without fluoride came to the market which contained, calcium sucrose phosphate (CaSP) was introduced as **ENAFIX (GROUP PHARMACEUTICALS)** in the Indian market as a remineralizing agent. This product usually decomposes to calcium, phosphate, and sucrose ions, thus resulting in increased rate of remineralization. [11]

Henceforth, no study compared the impact of strategies of delivery of calcium, phosphate with varying composition of fluoride ions, on the remineralizing potential of incipient carious lesions. Furthermore, there is paucity in literature regarding the study of the built up mineral products stability following acid challenge. Thus, this study would compare the effect of different calcium-phosphorous and fluoride delivery systems on enamel remineralization using surface micro-hardness, scanning electron microscopy and surface roughness methods.

AIM AND OBJECTIVES

AIM AND OBJECTIVES

AIM

The aim of the study was to compare and evaluate the remineralizing efficacy of different calcium-phosphate and fluoride based delivery vehicles on artificial caries like enamel lesions.

OBJECTIVES

- To evaluate the Surface Microhardness of artificial caries like enamel lesions after using artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix at the Baseline, 2 weeks of Remineralization, 4 weeks of Remineralization and after Final Acid challenge.
- To compare the Surface Microhardness of artificial caries like enamel lesions after using artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix at the Baseline, 2 weeks of Remineralization, 4 weeks of Remineralization and after Final Acid challenge.
- To evaluate the Surface Roughness of artificial caries like enamel lesions after using artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix at the Baseline, 2 weeks of Remineralization, 4 weeks of Remineralization and after Final Acid challenge.
- To compare the Surface Roughness of artificial caries like enamel lesions after using artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix at the Baseline, 2 weeks of Remineralization, 4 weeks of Remineralization and after Final Acid challenge.

- To evaluate the Qualitative Remineralizing potential of artificial caries like enamel lesions after using artificial saliva, Remin Pro, Tooth Mousse Plus and Enafix at the Baseline, 2 weeks of Remineralization, 4 weeks of Remineralization and after Final Acid challenge using Scanning Electron Microscope Images.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Reynolds EC et al (1997) carried out an in vitro study, to show that CPP-stabilized calcium phosphate solutions were shown to remineralize subsurface lesions in human third-molar enamel. Although most of the remineralizing solutions were supersaturated with respective amorphous and crystalline calcium phosphate phases, the solutions were stabilized by the CPP such that spontaneous precipitation of calcium phosphate did not occur. After a ten-day remineralization period, enamel lesions were sectioned, subjected to microradiography, and the mineral content was determined by microdensitometry. And the study concluded that the remineralizing capacity was greater for the solutions with the higher levels of CPP-stabilized free calcium and phosphate ions and so remineralization was not significantly correlated with either the CPP-bound ACP or the degrees of saturation for hydroxyapatite, octacalcium phosphate, or ACP. The study elaborated that CPP, by stabilizing calcium phosphate in solution, maintain high-concentration gradients of calcium and phosphate ions and ion pairs into the subsurface lesion and thus accounts for the effect high rates of enamel remineralization.[12]

Reynolds EC et al (2003) compared the ability of CPP-ACP, with that of other forms of calcium, to be retained in supragingival plaque and remineralize enamel subsurface lesions in their in situ study when delivered in a mouthrinse or sugar-free gum in randomized, double-blind trials. Thirty healthy adult subjects ranging in age from 22 to 44 yrs who volunteeted were included in the study. In the mouthrinse study, only the CPP-ACP-containing mouthrinse significantly increased plaque calcium and inorganic phosphate levels, and the CPP were immunolocalized to the surfaces of bacterial cells and the intercellular matrix. In the chewing gum studies, the gum containing the CPP-ACP, although not containing the most calcium per piece of gum, produced significantly the highest level of enamel remineralization independent of gum-

chewing frequency and duration. The CPP could be detected in plaque extracts 3 hours after subjects chewed the CPP-ACP-containing chewing gum. The results showed that CPP-ACP were superior to other forms of calcium in remineralizing enamel subsurface lesions.[10]

Pulido MT et al (2008) conducted an in-vitro study to evaluate the inhibition of demineralization in enamel sections produced by MI paste, fluoride and a combination of both, compared to that of artificial saliva and NaF 5000 ppm in a caries progression pH-cycling model. Twenty-one teeth were demineralized to create subsurface lesions in enamel (approximately 200 microns in depth). The teeth were sectioned and characterized using polarized-light-microscopy (PLM) device. A single section from each lesion was assigned to particular treatment group: Artificial saliva, NaF 5000 ppm (Prevident, Colgate), MI paste (Recaldent, GC America Inc), NaF 1100 ppm (Crest, Procter & Gamble) and NaF 1100 ppm plus MI paste respectively. The sections were covered with varnish except for an exposed window on the external surface of lesion and placed in a six-day pH-cycling model with two daily treatment applications of two minutes each. The sections were then characterized by PLM, and the lesion areas were measured using a digital image analysis system. Based on a paired sample t-test,a notable significant differences ($p<.05$) in percentage of change in lesion size were found between the high fluoride group and all the other groups. No significant difference was found between the artificial saliva and MI paste group, neither there was any significant difference between the NaF 1100 ppm, the combined application group or the MI paste group alone. In conclusion, the higher concentration of NaF (5000 ppm) showed reduced lesion progression to the greatest extent. The MI paste group did not show any effect on the inhibition of the lesion progression.[13]

Kumar VL et al (2008) investigated the efficacy of CPP-ACP containing Tooth Mousse on the remineralization of enamel lesions and to compare its efficacy to that of a fluoride-containing toothpaste and found that CPP-ACP containing Tooth Mousse remineralized initial enamel lesions and it showed a higher remineralizing potential when applied as a topical coating after the use of a fluoridated tooth- paste.[14]

Srinivasan N et al (2010) in their in-situ study compared the remineralization potential of pastes containing CPP–ACP and CPP–ACP with 900 ppm fluoride on the human enamel which is previously softened by a cola drink. Forty-five enamel specimens were obtained from human third molar teeth were eroded in a cola drink for period of 8 min and then attached to intra-oral devices worn by five volunteers. The specimens were then subjected to three different in situ remineralization protocols using: (1) CPP–ACP (Tooth mousse) (Group I), (2) CPP–ACP with 900 ppm fluoride (Group II) (Tooth mousse plus), and (3) saliva (Group III, control). Vicker's microhardness measurements were obtained at the baseline followed by demineralization and remineralization cycles. The study concluded that the CPP–ACP and CPP–ACP with 900 ppm fluoride substantially remineralized the softened enamel, with the CPP–ACP and fluoride combination showing significantly higher remineralization potential than CPP–ACP. [15]

Agnihotri Y et al (2012) investigated the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing tooth mousse on the remineralization of enamel lesions and compared its efficacy to fluoride containing tooth paste. The study concluded that CPP-ACP containing tooth mousse remineralized initial enamel lesions and showed a higher remineralizing potential than fluoridated toothpaste.[16]

Chapla et al (2013) evaluated the efficacy of CPP-ACPF and Sodium fluoride with tri-calcium phosphate on enamel remineralisation using Diagnodent and they concluded that three groups showed a statistically significant amount of remineralization, however, Clinpro 5000 showed marginally more amount of remineralisation than CPP-ACPF.[17]

Mehta R et al (2013) conducted an in-vitro study to evaluate and to compare the remineralization potential of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide-amorphous calcium fluoride phosphate (CPP-ACFP) on artificial white spot enamel lesions using the quantitative light fluorescence (QLF). A total of 45 caries-free extracted maxillary first premolars were embedded in the acrylic resin. The samples were then randomly divided into three groups namely control group, CPP-ACP group and CPP-ACFP group with 15 samples in each group. The samples of each group were subjected to demineralization process for a period of 96 h. The samples were then mounted in the artificial mouth model and subjected to remineralization and pH cycling for a total period of 21 days. QLF readings were recorded at the end of demineralization (1st, 7th, 14th and 21st day respectively) and were statistically analyzed. As compared with artificial saliva both CPP-ACP and CPP-ACFP produced significant amount of remineralization of the artificial on enamel white spot lesion, however when the remineralizing effect of CPP-ACP was compared with the remineralizing effect of CPP-ACFP there was no significant difference. Significant amount of remineralization was then produced by CPP-ACP and CPP-ACFP only after the 7th day. After the 14th day, the remineralization produced by both CPP-ACP and CPP-ACFP as compared to artificial saliva were non-significant. [18]

Patil N et al (2013) conducted an invitro study with an objective to find out the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), and tricalcium phosphate fluoride (TCP-F) in remineralizing enamel surface on which artificial caries lesion had been created earlier. The changes were then analyzed using DIAGNOdent® (KaVo) and scanning electron microscope (SEM). A total of 52 premolars and 24 molars were selected and then classified into four groups of 13 premolars and 6 molars in each: I (CPP-ACP), II (CPP-ACPF), III (TCP-F), and IV (artificial saliva). All the samples were assessed using DIAGNOdent at the baseline and after demineralization and then remineralization. Ten samples were randomly selected from each group baseline after demineralization and after remineralization for the surface evaluation using SEM. The study concluded that all the three experimental groups showed a statistically significant amount of remineralization. However, because of the added benefit of fluoride TCP-F and CPP-CPF showed marginally more amount of remineralization than CPP-ACP. [19]

Vanichvatana S et al (2013) tested the efficacy of two calcium phosphate pastes compared to that of fluoride toothpaste on remineralizing artificial caries *in situ* study using Clinpro, Tooth Mousse plus were used in conjugation with regular fluoridated tooth paste and regular colgate toothpaste alone and concluded that Clinpro Tooth Creme provided similar benefits to the fluoride toothpaste; however, no additional benefit of Tooth Mousse Plus was observed when used in conjunction with the fluoride toothpaste.[20]

Elkassas D et al (2014) conducted a study to assess enamel remineralization of various calcium-phosphate and fluoride delivery systems. Artificial caries lesions were created on 115 extracted human mandibular molars. Specimens were assigned according to remineralizing agent into five diffrent groups: G1: Control (artificial saliva), G2:

ClinproTM white varnish, G3: Relief, G4: Tooth Mousse Plus, G5: VanishTMXT. Surface micro-hardness (SMH), surface roughness (Ra) and surface topography by scanning electron microscope (SEM) were evaluated at baseline, after demineralization, after 2 and 4 weeks remineralization and after final acid challenge. Remineralizing agents containing different calcium-phosphate formulas and fluoride have increased remineralization potential when compared to artificial saliva. ClinproTM varnish presented the highest remineralization tendency with greatest resistance for acid challenge. Demineralized enamel showed the lowest SMH values. SEM analysis revealed mineralized coating on the surfaces which resists dissolution by acid challenge at variable degrees according to remineralization regimen that were applied. Remineralizing agents containing different calcium-phosphate formulas and fluoride have increased remineralization potential compared to that of artificial saliva. ClinproTM varnish presented the highest remineralization tendency with greatest resistance for the final acid challenge. [21]

Oliveira GM et al (2014) compared the remineralization effect on white spot lesions of casein phosphopeptide-amorphous calcium phosphate crème, or CPP-ACP (MI Past), 1.1% NaF dentifrice containing 5000 ppm of fluoride (ControlRX), or CPP-ACP crème with 900 ppm of fluoride (MI Paste Plus™) with that of a control and concluded that The 1.1% NaF dentifrice demonstrated overall greater remineralization ability than 10% CPP-ACP crème. However, the 1.1% NaF dentifrice was only as effective as the Control to reduce fluorescence loss. [22]

Shetty S et al (2014) conducted a study to evaluate the enamel remineralization after treatment with three different remineralizing agents using surface microhardness assessment. The in vitro study involves 50 enamel samples that were divided into five groups of 10 samples each. The positive control group consisted of intact enamel surface

and a negative control group consisted of demineralized enamel samples. All groups excluding the positive control group were then subjected to demineralization following which three of these groups were then remineralized using remineralizing agents (casein phosphopeptide amorphous calcium phosphate [CPP-ACP] [GC tooth mousse], casein phosphopeptide amorphous calcium phosphate with fluoride [CPP-ACPF] [GC tooth mousse plus], sodium fluoride [phos-flur]). The groups were so treated with remineralizing agents and were subjected to pH cycling over a period of 28 days. It was then followed with assessment of surface microhardness. The study concluded that there was an improved enamel remineralization in the group, remineralized using CPP-ACPF in comparison with the rest of the groups. [23]

Jeyarajan J et al 2015 conducted an *in vitro* study to find out the efficacy of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and casein phosphopeptide amorphous calcium phosphate fluoride (CPP-ACPF) in remineralizing enamel surface on which artificial caries lesion had been already created. The changes were analyzed using DIAGNOdent® (KaVo) and as well as scanning electron microscope (SEM). Ninety maxillary premolars were selected and divided into three groups of 30 teeth each: A (artificial saliva), B (CPP-ACP), and C (CPP-ACPF) respectvely. All the samples were assessed using DIAGNOdent at the baseline and also after demineralization and remineralization. Three samples were randomly selected from each group after remineralization for surface evaluation using SEM. All the three groups showed a statistically significant amount of remineralization, however, because of the added benefit of fluoride (NaF 0.2%), CPP-ACPF (Tooth Mousse-Plus) showed marginally more amount of remineralization than CPP-ACP (Tooth Mousse). [24]

Kaur G et al (2015) evaluated the effect of GC Tooth Mousse and Toothmin Tooth Cream on microhardness of bleached enamel. Both GC Tooth Mousse (Recaldent)

and Toothmin Tooth cream (Abbott Healthcare Pvt.Ltd) increased the microhardness of bleached enamel and Toothmin tooth cream proved to be a better agent for increasing microhardness, although difference is not significant. [25]

Krishnan S et al (2015) investigated the effect of McInnes bleaching agent on the microhardness of enamel before and after bleaching and to evaluate the effect of G C Tooth Mousse Plus and Toothmin on the bleached enamel surface for its microhardness using Vickers microhardness indenter and concluded that McInnes bleaching agent causes a decrease in the microhardness of enamel by causing enamel demineralization. GC Tooth mousse plus and toothmin used in this study caused increase in microhardness of the bleached enamel. Toothmin application led to more remineralization as compared to GC Tooth Mousse Plus even though it could not reach baseline level.[26]

Pishevar L et al (2015) conducted a study with aim to compare the effect of using Casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) paste, Remin-Pro and Fluoride Varnish on remineralization of surface lesions of enamel. The study included 60 intact premolars and molars that were divided into 6 groups. After primary DIAGNOdent value measurement and a four-day immersion in demineralizing solution, the DIAGNOdent value were then measured. Groups 1, 2 and 3 were treated by Fluoride varnish, CPP-ACP and Remin-Pro respectively, according to the manufacturer instruction and their DIAGNOdent value was then read. Groups 4, 5, and 6 were treated by Fluoride varnish, CPP-ACP and Remin-Pro for period of 1 month (8 hours a day), respectively, and their DIAGNOdent value was measured. Then the specimens of these three groups were demineralized and pH cycled and their DIAGNOdent values were then recorded. After a one-month treatment, the DIAGNOdent value significantly decreased in the 4, 5, and 6 groups. ANOVA test indicated that decrease mean value of DIAGNOdent value was significantly higher for Remin-Pro and CPP-ACP groups than

that of Fluoride varnish group, from entrance time to time of the study to re-demineralization stage. All the three materials showed a statistically significant amount of remineralization after repeated application but the CPP-ACP and Remin pro were more resistant to redemineralization as well as the pH cycling. [27]

Reddy BK et al (2015) evaluated the effect of remineralizing agents on the surface morphology and microhardness of bleached enamel using 2% Sodium Fluoride solution compared with CPP-ACP crème (GC Tooth Mousse, Recaldent and the study concluded that both the remineralizing agents used showed statistically significant increase in the microhardness of bleached enamel. [28]

Sandeep T et al (2015) evaluated the remineralization potential of various newer remineralizing agents such as Fluoride enriched Casein Phospho-peptide Amorphous calcium phosphate, Beta Tri- calcium Phosphate and Hydroxyapatite based cream on bleached enamel surface using Surface hardness test and finalized that among the remineralising agents tested in the study Hydroxyapatite based cream (Reminpro) was found to be marginally more effective than the CPP-ACPF (GC Tooth Mousse plus) and β-TCP (3M ESPE Clinpro) which was not statistically significant.[29]

Talaat DM et al (2015) evaluated the acid resistance of enamel subsurface lesions treated with casein phosphopeptide amorphous calcium phosphate fluoride (CPP ACPF) in fifty extracted primary molars with a standardized window on enamel that were immersed in a demineralizing solution for 72 hours to produce subsurface enamel lesions and later they were sectioned in a buccolingual direction; one half of the sample was treated with the remineralizing agent CPP-ACPF and the other half remained untreated (control group). After period of 10 days, the sample was evaluated quantitatively using energy dispersive X-ray spectroscopy. The treated samples were then reimmersed into the demineralizing solution for 72 hours and the mineral content

was then re-evaluated. The study concluded that the mean calcium content of the remineralized samples was significantly higher than that of the control group and so CPP-ACPF increased the resistance of enamel surfaces to further demineralization.[30]

Chokshi K et al (2016) conducted a study to compare and evaluate the remineralization potential of Fluoride Varnish, CPP-ACP Paste (Casein Phosphopeptide-Amorphous Calcium Phosphate) and fTCP Paste (functionalized Tricalcium Phosphate) using confocal microscope and concluded that Fluoride varnish showed the greatest remineralization potential of artificial carious lesions followed by CPP-ACP Paste and fTCP Paste respectively. [31]

Jayashankara CM et al (2016) compared the remineralizing effect of SHY-NM and Remin Pro tooth pastes on the microhardness of bleached enamel and evaluated the surface microhardness using Vickers microhardness tester and the study resulted The results revealed a significant decrease in enamel microhardness after bleaching procedure, the application of Remin Pro showed a significant increase in enamel microhardness whereas SHY-NM showed a marginal recovery in microhardness. The study concluded that Remin Pro exhibited a good recovery in enamel microhardness and can be ensured as a promising material for remineralisation of bleached enamel.[32]

Rafia KN et al (2016) compared the clinical efficacy of a dentrifice containing calcium sodium Phosphosilicate to a novel stabilized calcium sucrose phosphate dentrifice and to a placebo on dentinal hypersensitivity and concluded that the dentifrice containing 5% novamin (calcium sodium phosphosilicate) provides rapid and significantly more relief from dentin hypersensitivity in four weeks compared to a dentifrice containing calcium sucrose phosphate or a non-desensitizing dentifrice. [33]

Savas S et al (2016) evaluated the efficacy of CPP-ACP containing fluoride varnish on remineralizing white spot lesions by surface microhardness (SMH), quantitative light induced fluorescence digital(QLF-D), Energy Dispersive Spectroscopy

(EDS) and Laser fluorescence (LF-PEN) and found that CPP-ACP containing fluoride varnish remineralizes white spot lesions with a single application and seems suitable for clinical use. [34]

Singh P et al (2016) compared the protective effect of CPP-ACPF, TCP and Amine Fluoride on artificially eroded primary and permanent teeth using surface microhardness tester. The study finalized that primary and permanent enamel substrates reacted differently to different fluoridated compounds. TCP showed effective anticorrosive effect in primary teeth while there was no statistical difference among the agents in the anticorrosive treatment of permanent teeth. [35]

Tahmasbi et al (2016) compared the remineralizing potential of fluoride, MI Paste Plus (CPP-ACP-F) and Remin Pro for treatment of white spot lesions. Surface hardness was measured using a Vickers microhardness tester and the study concluded that MI Paste Plus, 0.05% NaF mouthwash were lesser were efficient for treatment of white spot lesions comparing to Reminpro.[36]

Ghani S et al (2017) evaluated the effects of application of CPP-ACP and NovaMin pastes on the cut dentin surface with and without retention of smear layer, on bond strength of the resin dentine interface, with an all-in-one adhesive. And they discovered that smear layer retained group and pretreatment with Novamin showed the highest shear bond strength when compared to the CPP-ACP Paste. [37]

Grewal N et al (2017) conducted a study to elucidate the enamel remineralization potential of saliva, cheese, casein phosphopeptide amorphous calcium phosphate (CPP-ACP) based synthetic agent, and fluoride toothpaste *In situ* study was carried out on sixty individuals who wore an intraoral appliance containing demineralised enamel slabs. Quantitative values of mineral content of slab were measured using energy dispersive Xray and qualitative changes in surface topography of

slab were seen under scanning electron microscope. A synergistic effect of fluoride containing toothpaste with intake of cheese could be a good enamel remineralization protocol. [38]

Kargul B et al (2017) evaluated the remineralization effects of the casein phosphopeptide-amorphous calciumphosphate (CPP-ACP) paste on the white spot lesions (WSLs) of the primary teeth and to assess its caries-prevention efficacy on early childhood caries (ECC) all subjects were instructed to use daily fluoridated toothpaste (500 ppm F- as NaF) and additionally applied a CPP-ACP containing paste on respective surfaces for 1 minute, twice a day. Baseline and final mineralization status were determined using a laser-induced infrared fluorescence (FL) device (DIAGNOdent. This 4-week clinical study have indicated that twice daily topical applications of CPP-ACP containing paste as an adjunct to a standard oral hygiene programme which includes fluoridated toothpaste, significantly improve the remineralisation of white spot lesions. The usage of CPP-ACP paste with Fluoride toothpaste could be effective for preventing demineralization and promoting remineralization of enamel subsurface lesions.[39]

Kamath P et al (2017) aimed to compare and evaluate the remineralization potential of commercially available agents containing nano-hydroxyapatite (nano-HA), casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), and Tricalcium phosphate (TCP) on artificially induced white spot lesions in primary teeth and they were analysed using DIAGNOdent readings and scanning electron microscope (SEM) energy dispersive X-ray (EDX) and they concluded that all test agents were comparable in their remineralization potential. [40]

Soares R et al (2017) evaluated the ability of Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride (CPP ACPF), Bioactive Glass (BAG), fluoride enhanced Hydroxyapatite (HA) gel and self-assembling peptide P11-4 to remineralise

artificial carious lesions in enamel in vitro using a 30 day pH cycling model through surface microhardness analysis and SEM and concluded that Self assembling peptide P11 -4 demonstrated promising results by effectively and significantly remineralising the enamel lesions as compared to other test agents. [41]

Beerens MW et al (2017) evaluated the long-term effect of MI paste plus versus a placebo paste on remineralization of enamel after fixed orthodontic treatment over a 12-month period and they concluded that there was no improvement in the subsurface lesions during the 1 year following debonding.[42]

Sharma A et al (2017) compared the remineralizing efficiency of the paste containing hydroxyapatite and casein phosphopeptide-amorphous calcium phosphate. The samples were analysed for surface hardness and mineral content and they concluded that both the agents used are effective in causing remineralization of enamel. Nano-hydroxyapatite is more effective as compared to Casein phosphopeptide-amorphous calcium phosphate, in increasing the Calcium and Phosphorus content of enamel, and this effect is more evident over a longer treatment period. [43]

Bakry AS et al (2018) compared the remineralization efficacy of using the MI paste plus to MI varnish using surface microhardness tester before and after acid challenge and found that MI paste plus has an increase in the remineralizing the enamel surface lesions that increases the efficacy of CPP-ACP in the suface remineralization. [44]

Gangrade A et al (2018) conducted a study to evaluate remineralizing efficacy of stannous fluoride (SnF2), casein phosphopeptide‑amorphous calcium phosphate with fluoride (CPP‑ACPF) and calcium sucrose phosphate (CaSP). He conducted the study in fifteen freshly sound maxillary premolars, extracted for orthodontic reasons. Enamel samples were embedded in a self‑cure acrylic with their enamel surface exposed, and were then randomly divided into five groups of 10 samples each. The samples were then

assigned into following groups: (Group A Demineralized and treated with stannous fluoride (Colgate Gel Kam) SnF2 Group B Demineralized and treated witih calcium sucrose phosphate (EnaFix) CaSP Group C Demineralized and treated with CPP-ACPF (GC Tooth Mousse Plus) CPP-ACPF. (All the samples of Groups A, B, C, and E were then demineralised the samples in Groups A, B, and C were treated with respective remineralizing agents at every 24 h for 7 days Group C was not remineralized. After seven cycles of remineralization, the Surface Microhardness of the specimens were determined using Vickers microhardness testing machine. The study concluded that complete remineralization did not occur within 7 days. SnF2 showed the highest potential for remineralization followed by CaSP and CPP-ACPF. [45]

Hedge MN et al 2018 conducted a study to quantitatively evaluate the remineralization potential of casein phosphopeptide-amorphous calcium phosphate paste on enamel subsurface lesions using scanning electron microscopy with energy dispersive X-ray analysis (SEM-EDX). Ninety enamel specimens were then prepared from extracted human molars. All the specimens were evaluated for mineral content (% weight) using SEM-EDX. The specimens were then placed in demineralizing solution for period of four days to produce artificial carious lesions. The mineral content (calcium/phosphorus ratios, Ca/P ratios) was then remeasured using SEM-EDX. The specimens were then randomly assigned to five study groups and one control group of 15 specimens per group. Except for the control group, all group specimens were then incubated in remineralizing paste (CPP-ACP paste) for 7, 14, 21, 28, and 35 days twice daily for three minutes. The control group received no treatment with remineralizing paste. All the 90 specimens were then stored in artificial saliva at 37°C. After remineralization, the mineral content (% weight) of the samples was measured using SEM-EDX. The study concluded that groups showed very highly significant differences

between Ca/P ratios of the demineralized and remineralized samples and there was no significant difference seen in the control group and CPP-ACP paste could significantly remineralize the artificial enamel subsurface lesions *in vitro*: the remineralizing rates increasing with the time for which the samples were kept in the respective remineralizing paste.[46]

Heravi F et al (2018) investigated the effectiveness of two remineralizing creams on regression of white spot lesions using a 12-week regimen of a cream containing casein phosphopeptide-amorphous calcium phosphate and fluoride (MI Paste Plus) compared with a 12-week regimen of a cream containing hydroxyapatite and fluoride (Remin Pro) compared with a usual home care (control).

The application of either MI Paste Plus or Remin Pro was effective in reducing the area, increasing the mineral content and improving the appearance of demineralized enamel, indicating that these products could be recommended for managing white spot lesions. [47]

Gade V et al (2018) evaluated the remineralization efficacy of two different remineralizing agents. Forty enamel samples were taken, and they were so divided into four groups, the demineralization was carried out with Groups A, B, and C. Remineralization was carried out with Groups A and B period of 7 continuous days using casein phosphopeptide‐amorphous calcium phosphate with fluoride (CPP‐ACPF) and calcium sucrose phosphate (CaSP). Group D was kept as positive control with intact enamel without any surface treatment, whereas Group C was kept as negative control with surface demineralization of the enamel. Microhardness testing was done using Vickers microhardness tester after 7‐day remineralization cycle and they concluded that Both remineralizing agents showed surface remineralization. [48]

Lata S et al (2018) conducted a study with the aim of evaluating the remineralization potential of fluoride and ACP-CPP and the combination of ACP-CPP and fluoride on early enamel lesions created in the enamel blocks of human premolars Fifteen intact carious free human premolars were selected and the coronal part of each tooth was sectioned into four parts to make 4 enamel blocks. The baseline SMH (surface microhardness) was measured for all the enamel specimens using the Vickers microhardness (VHN) testing machine. Artificial enamel carious lesions were created by inserting the specimens in the demineralization solution for period of 3 consecutive days. The SMH of the demineralised specimens was then evaluated. Then the four enamel sections of each tooth samples were subjected to various surface treatments , i.e. Group 1- Fluoride varnish, Group 2- ACP-CPP cream, Group 3- Fluoride + ACP-CPP & Group 4- Control (No surface treatment) respectively. A caries progression test (pH cycling) was carried out, which consisted of alternative demineralization (3hours) and remineralization with artificial saliva (21 hours) for five consecutive days. After pH cycling again SMH of each specimen was then assessed to evaluate the remineralization potential of each surface treatment agent. Then finally to asses the remineralization potential of various surface treatments at the subsurface level, each enamel specimen was longitudinally sectioned through the centre to expose their subsurface enamel area. Cross-sectional microhardness (CSMH) was evaluated to assess any subsurface remineralization. The study concluded that ACP-CPP cream is effective, but to a lesser extent than fluoride in remineralizing early enamel caries at surface level. Combination of fluoride and ACP-CPP does not provide any additive remineralization potential compared to fluoride alone. Fluoride, ACP-CPP and their combination are not effective in remineralizing the early enamel caries at their subsurface level. [49]

Reham M et al (2018) conducted a study to determine the effect of fluoride gel, Remin Pro, and GC tooth mousse plus in changing surface roughness of enamel after bleaching procedures. Human dental third molars were selected for the study. The 38% hydrogen peroxide in‑office bleaching protocols were performed on the specimens and they were randomly divided into three groups (n = 10 samples per group) according to the remineralizing agent used: group 1: using fluoride gel, group 2: using Remin Pro, and group 3: using GC tooth mousse plus. Measurements of surface roughness were carried out using a contact stylus profilometer before bleaching, after bleaching, and after remineralization and finally the Datas were analyzed using analysis of variance and Tukey's test. There was a highly significant difference of enamel surface roughness measurements among initial, after bleaching, and final regarding the three tested groups (P < 0.001). There was no significant difference among the three tested groups in relation to enamel surface roughness before and after bleaching and it concluded that GC tooth mousse plus and Remin Pro are more effective in reducing enamel surface roughness after bleaching than the regular fluoride product. [50]

MATERIALS & METHODS

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ARMAMENTARIUM

- 92 Mandibular Molars
- Saturated Thymol
- Steriomicroscope (Nikon, Japan)
- Custom-made plastic cylindrical molds (1in x.5in)
- Self-cure Acrylic Resin
- Acid resistant nail varnish
- Applicator Tip
- Wax Sheet
- Demineralizing Solution (**Pulido et al 2008)** [13]
- Artificial Saliva **(Torres et al. 2012)** [51]
- Deionized Water
- Remin Pro (VOCO)
- Tooth Mousse Plus (GC INDIA)
- Enafix (GROUP PHARMACEUTICALS)
- Vickers Hardness Tester (FIE)
- Scanning Electron Microscope (ZEISS)
- Contact Profilometer (mityuto)

SPECIMENS PREPARATION

Total of 136 extracted human mandibular molars are selected free from clinically visible abnormality were stored in an aqueous solution of saturated thymol for a period of 2 weeks. The teeth were thoroughly rinsed and examined under the stereomicroscope (**Nikon, Japan**), to elicitate the presence of any tooth with defects, erosions, or micro-

cracks on their enamel surfaces or visible stains. Any visible or detectable caries or any white spot lesions were excluded unless those didn't involve their buccal surface and total of 92 mandibular molars were selected for the study (Fig 1).

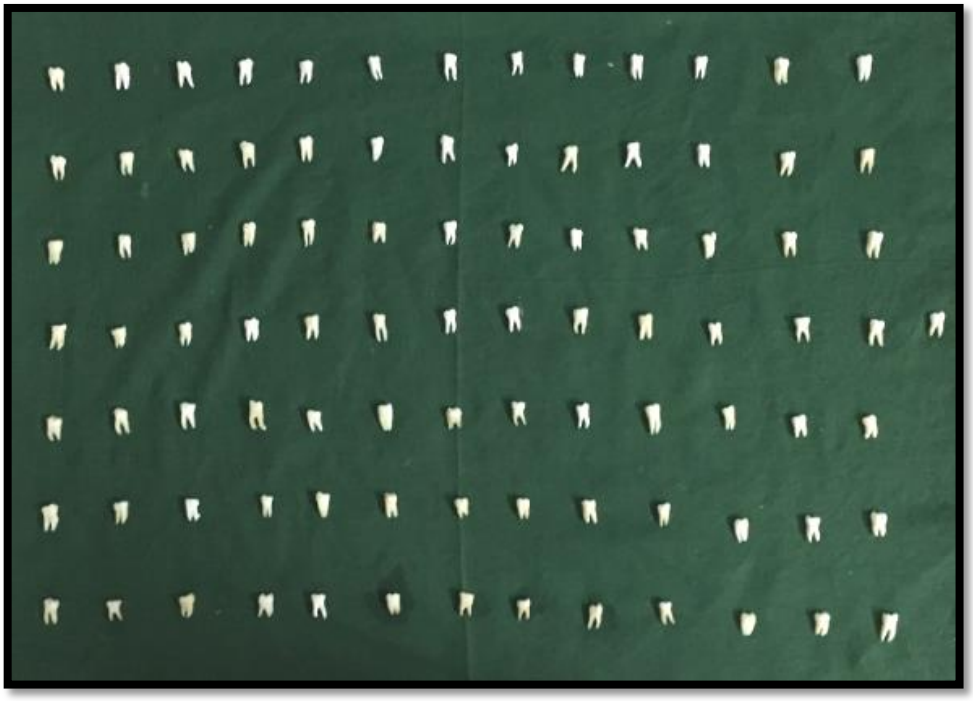


FIG 1: 92 MANDIBULAR MOLARS

Custom-made plastic cylindrical molds (1inch x 1inch) were prepared and self-cured acrylic resin was poured on each of the plastic mold. Each buccal of the mandibular molar section was horizontally mounted in acrylic resin and cured overnight.

An acid resistant nail varnish was applied using applicator tip around the exposed enamel surface on the buccal aspect of the molars, leaving four equal overtures of wax sheet which is approximately (2 mm x 2 mm) each (Fig 2). And the wax sheets are removed later once the nail varnish is set (Fig 2).

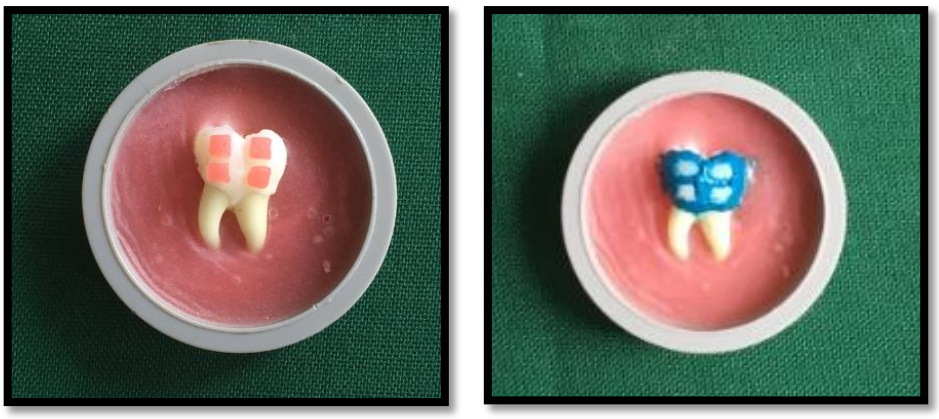


FIG 2: CREATING WINDOWS ON BUCCAL SURFACE

DEMINERALIZATION

The proposal suggested by **Pulido et al 2008** [13] has been followed for creating artificial caries like lesions, by individually immersing acrylic-mounted enamel specimens in continuously stirred, daily renewed demineralization solution. The solution contains 2.2 mM CaCl2_2H2O, 2.2mM KH2PO4, 0.05M acetic acid and pH adjusted to 4.4 with 10 MKOH (Fig 3). The mounted molars were kept in this solution for 5 days until a uniform white spot lesion were created on the surface of the window. Enamel specimens were washed carefully and then the first window was coated with acid resistant nail varnish to act as control for the intial demineralization (Fig 4).

FIG 3: SAMPLES IMMERSED IN DEMINERALIZING AGENT

FIG 4: FIRST WINDOW CLOSURE AFTER INTIAL DEMINERALIZATION

PREPARATION OF ARTIFICIAL SALIVA

Artificial saliva was prepared according to **Torres et al. 2012**[51] formula that comprises of 22.1mM hydrogen carbonate, 16.1mM potassium, 14.5mM sodium, 2.6 mM hydrogen phosphate, 0.8mMboric acid, 0.7mMcalcium, 0.2mM thiocyanate, and 10.2mM magnesium. The pH was adjusted between 7.4 and 7.8.

REMINERALIZATION

All the samples were stored in deionized water until further use. A total of ninenty two enamel samples embedded in acrylic slabs were produced, and were then randomly divided into five groups of 23 samples each for further remineralization.

All the specimens were randomly divided into four following groups :

GROUP 1: Artificial Saliva (Control group): No treatment was given to the enamel surface and specimens were kept in artificial saliva which was renewed every day.

GROUP 2 : Remin Pro (VOCO) – Calcium, Phosphate, Xylitol and 1,450ppm of Fluoride. (**HA_p** – Hydroxyapatite).

GROUP 3: Tooth Mousse Plus (GC INDIA) – **C**aesin **P**hos**P**hopeptide **A**morphous **C**alcium **P**hosphate containing 900ppm of **F**luoride.

GROUP 4: Enafix (GROUP PHARMACEUTICALS) – Calcium **S**ucrose **P**hosphate and amorphous calcium phosphate.

The samples of each group were differentiated by making vent holes at different areas of the acrylic mold (Fig 5).

GROUP 1 (Absence of vent hole in the mold)

GROUP 2 (Two parallel vent hole are present in the right side of the mold)

GROUP 3 (A single vent hole is present in the right side of the mold)

GROUP 4 (A single vent hole is present in the left side of the mold)

FIG 5: REMINERALIZING AGENTS AND VENT HOLES ON ACRYLIC TOOTH MOLD – TO DIFFERENTIATE BETWEEN THE GROUPS

APPLICATION OF TEST AGENTS

The samples in each group were treated with the respective remineralizing agent that were continuously applied onto the remaining 3 windows of the tooth surface with the help of a disposable cotton applicator tip for 3 minutes once in every 24 hours for a period of 4weeks (Fig 6). Samples after application of the remineralizing agents were then washed with deionized water and then placed in artificial saliva, that was changed once in every 24 hours until the final acid challenge.

FIG 6: TEST AGENT APPLICATION

Remineralization process was continued for a period of 2 weeks and the second window was coated with acid resistant nail varnish, the remaining two windows were continued remineralization and the third window was closed after a period of 4 weeks.

FIG 7: SECOND AND THIRD WINDOW CLOSURE

After these periods, the samples were immersed again in to the previously described demineralizing solution for a period of 5 days to evaluate the acid resistance of the treated surfaces and the fourth window was coated with acid reisitant nail varnish.

The 23 specimens of each group were then divided into three groups and tested by the following methods: assessment of the surface micro-hardness (n = 10), scanning electron microscopy examination (n = 3) and assessment of the surface roughness (n = 10). The nail varnish was peeled off from all of the specimens carefully before the analysis.

FIG 8: IMMERSION OF SAMPLES IN THE DEMINERALIZATION AGENT; FOURTH WINDOW CLOSURE

ASSESSMENT OF SURFACE MICRO-HARDNESS (SMH)

Enamel surface micro-hardness was measured at baseline of sound untreated enamel, after intial demineralization, after 2 weeks remineralization, after 4 weeks remineralization and after being subjected to final acid challenge. The surface microhardness of the specimens were determined using FIE (Fuel Instruments and engineering Pvt. Limited) microhardness tester. A load of 100 g were exercised steadily to the surface of specimens for 10sec using Vickers elongated diamond pyramid indenter. Four indentations were placed on the surface of each window and the average value was considered for each specimen. All readings were performed by the same examiner using the same calibrated machine (Fig 9).

FIG 9: SAMPLE ANALYSED IN VICKERS HARDNESS TESTER (FIE)

SCANNING ELECTRON MICROSCOPE EXAMINATION (SEM)

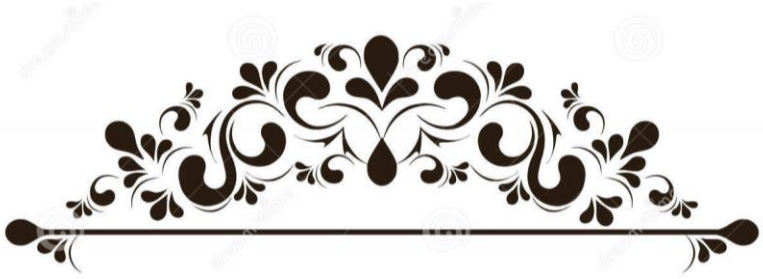
For the SEM examination, three specimens in each group were treated and then air dried. Each specimen was mounted on SEM metal stud. They were examined under Scanning Electron Microscope (EVO 18 RESEARCH; ZEISS) (Fig 10). SEM photomicrographs were captured at 1000x and 500x magnifications in order to gain their qualitative results (Fig 12).

FIG 10: SAMPLE ANALYSED IN SCANNING ELECTRON MICROSCOPE (ZEISS)

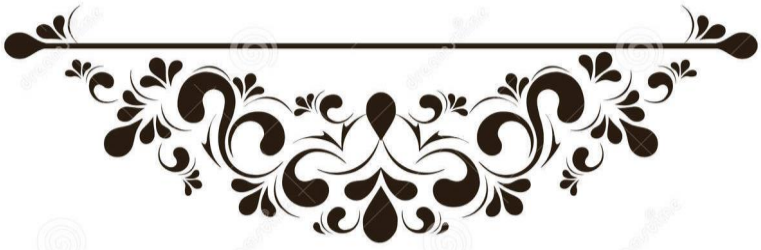
ASSESSMENT OF SURFACE ROUGHNESS (R_a)

This method tends to fulfil the need for the quantitative characterization of surface topography with contact. Specimens were examined using Mityuto SJ 410 Contact profilometer (Fig 11). Tip angle of 60^0, diameter of 1.2 microns and force exerted was .75mN. Calibration was made by running the pointed intender along each window of the samples and the roughness values are expressed in μm.

FIG 11: SAMPLE ANALYSED IN CONTACT PROFILOMETER (Mityuto)



RESULTS



RESULTS

After evaluating the surface microhardness and surface roughness of the four groups, the data were subjected to one way ANOVA test for equality of means and 95% confidence interval of the differences [P<0.05] statistical analysis to identify the statistical significance of groups. Post hoc test was used to confirm where the differences occurred between the four groups.

ENAMEL SURFACE MICROHARDNESS

The mean (_SD) SMH values of all the surfaces treated at different enamel treatment points are shown in Table 1 (ANOVA TEST) and Table 2 (TURKEY POST HOC TEST) with graphic representation in GRAPH 1. All the groups presented significantly highest SMH at the baseline ($p < 0.05$). The demineralized enamel surface of all the specimens showed significant reduction in SMH values ($p < 0.05$). The SMH values of all treated groups demonstrated a marked significance in the SMH values after 2 and 4 weeks of application of the various remineralization regimen.

All of the groups showed significant reduction in the SMH values indicating mineral loss as exposed to acid challenge. SMH values in the Group 3 were significantly the highest, early after 2 weeks remineralization that has been elicited in the graphic representation Graph 1 followed by Group 2 and Group 4 respectively and least SMH value was found in Group 1. And even after 4 weeks of remineralization highest SMH value was found in Group 3. In addition, after the 4 weeks remineralization, the results between Group 4 and Group 2 ($p > 0.05$) were not statistically significant. After exposure to acid challenge, Group 4 showed statistically significant higher SMH values followed by Group 3 and Group 2 and the least SMH value were found in Group 1.

TABLE 1: SURFACE MICROHARDNESS ONE-WAY ANOVA RESULTS

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
SMH - BASELINE	GROUP 1	10	286.473000	22.3912017	7.0807197	270.455299	302.490701	250.1600	318.2600
	GROUP 2	10	276.912000	12.9196902	4.0855648	267.669810	286.154190	260.5100	294.5600
	GROUP 3	10	275.881000	30.0574130	9.5049886	254.379222	297.382778	198.6500	301.1600
	GROUP 4	10	262.077000	30.6722336	9.6994119	240.135406	284.018594	210.1600	312.8100
	Total	40	275.335750	25.6399184	4.0540271	267.135706	283.535794	198.6500	318.2600
SMH - INTIAL DEMINERALIZATION	GROUP 1	10	189.4670	6.35365	2.00920	184.9219	194.0121	180.31	198.46
	GROUP 2	10	185.7240	13.95051	4.41154	175.7444	195.7036	150.81	200.16
	GROUP 3	10	183.0260	24.70533	7.81251	165.3529	200.6991	160.41	250.26
	GROUP 4	10	172.3240	16.31932	5.16062	160.6499	183.9981	150.21	200.18
	Total	40	182.6352	17.26996	2.73062	177.1120	188.1585	150.21	250.26
SMH - 2 WEEKS OF REMINERALIZATION	GROUP 1	10	164.349000	9.0625560	2.8658318	157.866038	170.831962	152.6500	182.4500
	GROUP 2	10	204.276000	8.7316070	2.7611766	198.029785	210.522215	190.1200	214.6500
	GROUP 3	10	215.491000	23.9893228	7.5860900	198.330072	232.651928	198.4100	280.2100
	GROUP 4	10	196.693000	16.7865754	5.3083812	184.684607	208.701393	170.5100	219.4300
	Total	40	195.202250	24.6107884	3.8913073	187.331338	203.073162	152.6500	280.2100
SMH - 4 WEEKS OF REMINERALIZATION	GROUP 1	10	184.860000	6.3537128	2.0092204	180.314828	189.405172	172.4500	194.5400
	GROUP 2	10	223.817000	11.5860587	3.6638335	215.528833	232.105167	200.5600	240.1200
	GROUP 3	10	244.170000	25.7398787	8.1396643	225.756800	262.583200	210.5100	296.4100
	GROUP 4	10	215.482000	14.5350953	4.5964007	205.084219	225.879781	190.8500	240.6800
	Total	40	217.082250	26.6194085	4.2088980	208.568950	225.595550	172.4500	296.4100
SMH - FINAL ACID	GROUP 1	10	170.385000	10.0591840	3.1809933	163.189093	177.580907	155.8500	188.5500
	GROUP 2	10	182.628000	11.4329259	3.6154086	174.449377	190.806623	165.4200	194.5200
	GROUP 3	10	187.107000	23.4493473	7.4153347	170.332347	203.881653	155.6500	240.1600
	GROUP 4	10	193.488000	22.0549001	6.9743718	177.710875	209.265125	160.1200	222.8100
	Total	40	183.402000	19.1275498	3.0243312	177.284713	189.519287	155.6500	240.1600

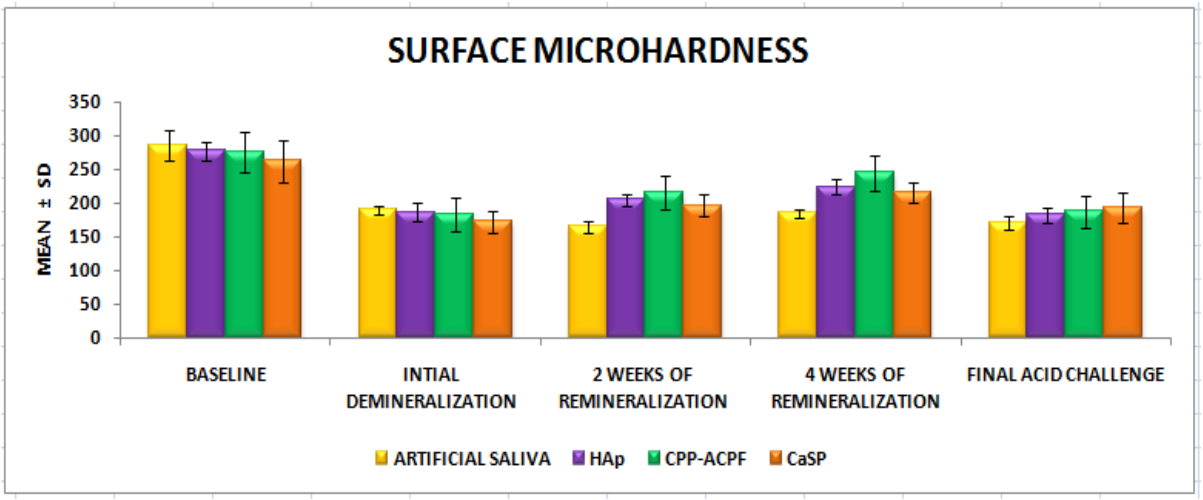
		Sum of Squares	df	Mean Square	F	Sig.
SMH - BASELINE	Between Groups	3026.147	3	1008.716	1.606	.205
	Within Groups	22612.665	36	628.130		
	Total	25638.811	39			
SMH - INTIAL DEMINERALIZATION	Between Groups	1626.877	3	542.292	1.951	.139
	Within Groups	10004.934	36	277.915		
	Total	11631.812	39			
SMH - 2 WEEKS OF REMINERALIZATION	Between Groups	14481.117	3	4827.039	19.011	.000
	Within Groups	9140.828	36	253.912		
	Total	23621.945	39			
SMH - 4 WEEKS OF REMINERALIZATION	Between Groups	18199.373	3	6066.458	23.145	.000
	Within Groups	9435.751	36	262.104		
	Total	27635.123	39			
SMH - FINAL ACID CHALLENGE	Between Groups	2854.958	3	951.653	3.002	.043
	Within Groups	11413.705	36	317.047		
	Total	14268.663	39			

TABLE 2: SURFACE MICROHARDNESS TUKEY POST HOC RESULTS

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
SMH - BASELINE	GROUP 1	GROUP 2	9.5610000	11.2082967	.829	-20.625489	39.747489
		GROUP 3	10.5920000	11.2082967	.781	-19.594489	40.778489
		GROUP 4	24.3960000	11.2082967	.149	-5.790489	54.582489
	GROUP 2	GROUP 1	-9.5610000	11.2082967	.829	-39.747489	20.625489
		GROUP 3	1.0310000	11.2082967	1.000	-29.155489	31.217489
		GROUP 4	14.8350000	11.2082967	.554	-15.351489	45.021489
	GROUP 3	GROUP 1	-10.5920000	11.2082967	.781	-40.778489	19.594489
		GROUP 2	-1.0310000	11.2082967	1.000	-31.217489	29.155489
		GROUP 4	13.8040000	11.2082967	.611	-16.382489	43.990489
	GROUP 4	GROUP 1	-24.3960000	11.2082967	.149	-54.582489	5.790489
		GROUP 2	-14.8350000	11.2082967	.554	-45.021489	15.351489
		GROUP 3	-13.8040000	11.2082967	.611	-43.990489	16.382489
SMH - INTIAL DEMINERALIZATION	GROUP 1	GROUP 2	3.74300	7.45540	.958	-16.3361	23.8221
		GROUP 3	6.44100	7.45540	.823	-13.6381	26.5201
		GROUP 4	17.14300	7.45540	.117	-2.9361	37.2221
	GROUP 2	GROUP 1	-3.74300	7.45540	.958	-23.8221	16.3361
		GROUP 3	2.69800	7.45540	.984	-17.3811	22.7771
		GROUP 4	13.40000	7.45540	.291	-6.6791	33.4791
	GROUP 3	GROUP 1	-6.44100	7.45540	.823	-26.5201	13.6381
		GROUP 2	-2.69800	7.45540	.984	-22.7771	17.3811
		GROUP 4	10.70200	7.45540	.486	-9.3771	30.7811
	GROUP 4	GROUP 1	-17.14300	7.45540	.117	-37.2221	2.9361
		GROUP 2	-13.40000	7.45540	.291	-33.4791	6.6791
		GROUP 3	-10.70200	7.45540	.486	-30.7811	9.3771
SMH - 2 WEEKS OF REMINERALIZATION	GROUP 1	GROUP 2	-39.9270000*	7.1261757	.000	-59.119410	-20.734590
		GROUP 3	-51.1420000*	7.1261757	.000	-70.334410	-31.949590
		GROUP 4	-32.3440000*	7.1261757	.000	-51.536410	-13.151590
	GROUP 2	GROUP 1	39.9270000*	7.1261757	.000	20.734590	59.119410
		GROUP 3	-11.2150000	7.1261757	.406	-30.407410	7.977410
		GROUP 4	7.5830000	7.1261757	.713	-11.609410	26.775410
	GROUP 3	GROUP 1	51.1420000*	7.1261757	.000	31.949590	70.334410
		GROUP 2	11.2150000	7.1261757	.406	-7.977410	30.407410
		GROUP 4	18.7980000	7.1261757	.057	-.394410	37.990410
	GROUP 4	GROUP 1	32.3440000*	7.1261757	.000	13.151590	51.536410
		GROUP 2	-7.5830000	7.1261757	.713	-26.775410	11.609410
		GROUP 3	-18.7980000	7.1261757	.057	-37.990410	.394410

SMH - 4 WEEKS OF REMINERALIZATION	GROUP 1	GROUP 2	-38.9570000*	7.2402237	.000	-58.456567	-19.457433
		GROUP 3	-59.3100000*	7.2402237	.000	-78.809567	-39.810433
		GROUP 4	-30.6220000*	7.2402237	.001	-50.121567	-11.122433
	GROUP 2	GROUP 1	38.9570000*	7.2402237	.000	19.457433	58.456567
		GROUP 3	-20.3530000*	7.2402237	.038	-39.852567	-.853433
		GROUP 4	8.3350000	7.2402237	.661	-11.164567	27.834567
	GROUP 3	GROUP 1	59.3100000*	7.2402237	.000	39.810433	78.809567
		GROUP 2	20.3530000*	7.2402237	.038	.853433	39.852567
		GROUP 4	28.6880000*	7.2402237	.002	9.188433	48.187567
	GROUP 4	GROUP 1	30.6220000*	7.2402237	.001	11.122433	50.121567
		GROUP 2	-8.3350000	7.2402237	.661	-27.834567	11.164567
		GROUP 3	-28.6880000*	7.2402237	.002	-48.187567	-9.188433
SMH - FINAL ACID CHALLENGE	GROUP 1	GROUP 2	-12.2430000	7.9630066	.426	-33.689186	9.203186
		GROUP 3	-16.7220000	7.9630066	.172	-38.168186	4.724186
		GROUP 4	-23.1030000*	7.9630066	.031	-44.549186	-1.656814
	GROUP 2	GROUP 1	12.2430000	7.9630066	.426	-9.203186	33.689186
		GROUP 3	-4.4790000	7.9630066	.943	-25.925186	16.967186
		GROUP 4	-10.8600000	7.9630066	.530	-32.306186	10.586186
	GROUP 3	GROUP 1	16.7220000	7.9630066	.172	-4.724186	38.168186
		GROUP 2	4.4790000	7.9630066	.943	-16.967186	25.925186
		GROUP 4	-6.3810000	7.9630066	.853	-27.827186	15.065186
	GROUP 4	GROUP 1	23.1030000*	7.9630066	.031	1.656814	44.549186
		GROUP 2	10.8600000	7.9630066	.530	-10.586186	32.306186
		GROUP 3	6.3810000	7.9630066	.853	-15.065186	27.827186

GRAPH 1: SURFACE MICROHARDNESS



ENAMEL SURFACE ROUGHNESS

The mean (_SD) R_a values of surfaces at different enamel treatment points are presented in Table 3 (ANOVA TEST) and Table 4 (TUKEY POST HOC TEST) with graphic representation in Graph 2. At the baseline measure, the mean Ra values of specimens were low. After demineralization, significant increase in Ra values was evident in all the groups tested ($p < 0.05$).

After 2 weeks remineralization, all experimental groups showed significant reduction in their respective R_a values. R_a values in the Group 3 were significantly lowest, early after 2 weeks of remineralization followed by Group 2 and Group 4 respectively and highest R_a values were found in Group 1. After 4 weeks of remineralization, further significant reduction in the R_a values was evident in Group 3, Group 2 and Group 4; however, no significant difference in R_a values were evident in Group 1. Interestingly, Group 4 and Group 2 group showed similar reduction in R_a values by 4 weeks remineralization next to Group 3($p > 0.05$). Following the exposure to acid challenge, all tested groups showed significant increase in R_a values when compared to 4 weeks remineralization. After exposure to acid challenge, Group 4 showed statistically significant lowest R_a values followed by Group 3 and Group 2 and the highest R_a value was found in Group 1.

Comparing between the remineralizing agents examined at different intervals, it was evident that Group 4 showed significantly lowest R_a values after 2 weeks of remineralization mean while, they showed significantly the lowest R_a values after exposure to acid challenge.

TABLE 3: SURFACE ROUGHNESS ANOVA RESULTS

		N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Ra - BASELINE	GROUP 1	10	.52170	.058965	.018646	.47952	.56388	.416	.589
	GROUP 2	10	.49140	.058138	.018385	.44981	.53299	.412	.582
	GROUP 3	10	.51470	.041679	.013180	.48488	.54452	.469	.591
	GROUP 4	10	.45800	.077342	.024458	.40267	.51333	.313	.542
	Total	40	.49645	.063231	.009998	.47623	.51667	.313	.591
Ra - INTIAL DEMINERALIZATION	GROUP 1	10	1.47390	.264458	.083629	1.28472	1.66308	1.014	1.916
	GROUP 2	10	1.59040	.148527	.046968	1.48415	1.69665	1.462	1.894
	GROUP 3	10	1.65530	.190369	.060200	1.51912	1.79148	1.316	1.912
	GROUP 4	10	1.56430	.179997	.056920	1.43554	1.69306	1.316	1.926
	Total	40	1.57098	.203514	.032178	1.50589	1.63606	1.014	1.926
Ra - 2 WEEKS OF REMINERALIZATION	GROUP 1	10	1.44420	.169796	.053694	1.32273	1.56567	1.314	1.891
	GROUP 2	10	1.27100	.078881	.024944	1.21457	1.32743	1.148	1.414
	GROUP 3	10	1.17530	.073615	.023279	1.12264	1.22796	1.121	1.345
	GROUP 4	10	1.32060	.162409	.051358	1.20442	1.43678	1.020	1.542
	Total	40	1.30278	.158310	.025031	1.25214	1.35341	1.020	1.891
Ra - 4 WEEKS OF REMINERALIZATION	GROUP 1	10	1.41070	.134148	.042421	1.31474	1.50666	1.242	1.718
	GROUP 2	10	1.22880	.130033	.041120	1.13578	1.32182	.981	1.341
	GROUP 3	10	1.07350	.158113	.050000	.96039	1.18661	.864	1.321
	GROUP 4	10	1.19370	.052529	.016611	1.15612	1.23128	1.124	1.258
	Total	40	1.22668	.171582	.027129	1.17180	1.28155	.864	1.718
Ra - FINAL ACID CHALLENGE	GROUP 1	10	1.47020	.212744	.067276	1.31801	1.62239	1.145	1.810
	GROUP 2	10	1.36020	.082367	.026047	1.30128	1.41912	1.241	1.456
	GROUP 3	10	1.31940	.129191	.040854	1.22698	1.41182	1.110	1.521
	GROUP 4	10	.92370	.137286	.043414	.82549	1.02191	.718	1.216
	Total	40	1.26838	.252877	.039983	1.18750	1.34925	.718	1.810

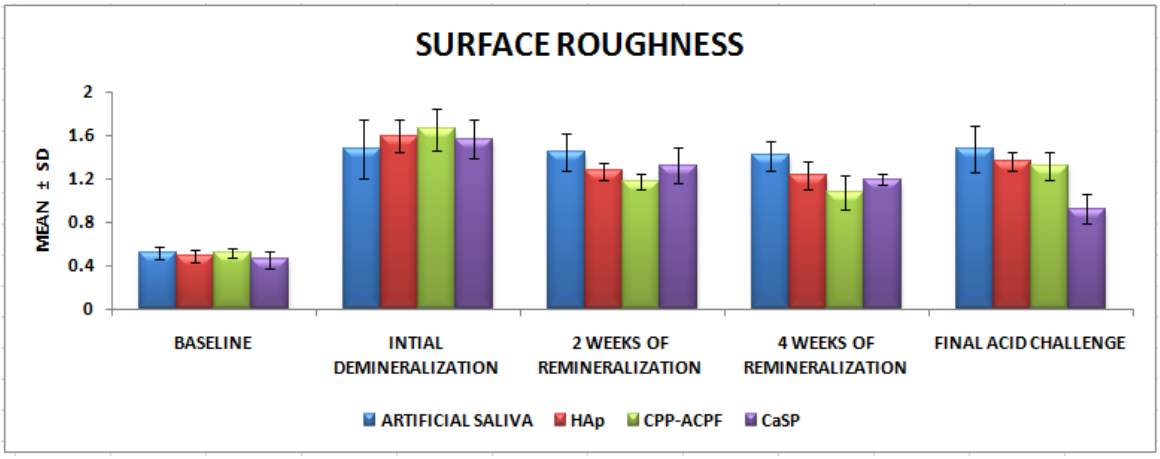
		Sum of Squares	df	Mean Square	F	Sig.
Ra - BASELINE	Between Groups	.025	3	.008	2.264	.098
	Within Groups	.131	36	.004		
	Total	.156	39			
Ra - INTIAL DEMINERALIZATION	Between Groups	.170	3	.057	1.407	.256
	Within Groups	1.446	36	.040		
	Total	1.615	39			
Ra - 2 WEEKS OF REMINERALIZATION	Between Groups	.376	3	.125	7.495	.001
	Within Groups	.602	36	.017		
	Total	.977	39			
Ra - 4 WEEKS OF REMINERALIZATION	Between Groups	.584	3	.195	12.430	.000
	Within Groups	.564	36	.016		
	Total	1.148	39			
Ra - FINAL ACID CHALLENGE	Between Groups	1.706	3	.569	25.967	.000
	Within Groups	.788	36	.022		
	Total	2.494	39			

TABLE 4: SURFACE ROUGHNESS TUKEY POST HOC RESULTS

Dependent Variable	(I) GROUP	(J) GROUP	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Ra - BASELINE	GROUP 1	GROUP 2	.030300	.026996	.678	-.04241	.10301
		GROUP 3	.007000	.026996	.994	-.06571	.07971
		GROUP 4	.063700	.026996	.104	-.00901	.13641
	GROUP 2	GROUP 1	-.030300	.026996	.678	-.10301	.04241
		GROUP 3	-.023300	.026996	.824	-.09601	.04941
		GROUP 4	.033400	.026996	.608	-.03931	.10611
	GROUP 3	GROUP 1	-.007000	.026996	.994	-.07971	.06571
		GROUP 2	.023300	.026996	.824	-.04941	.09601
		GROUP 4	.056700	.026996	.172	-.01601	.12941
	GROUP 4	GROUP 1	-.063700	.026996	.104	-.13641	.00901
		GROUP 2	-.033400	.026996	.608	-.10611	.03931
		GROUP 3	-.056700	.026996	.172	-.12941	.01601
Ra - INTIAL DEMINERALIZATION	GROUP 1	GROUP 2	-.116500	.089621	.569	-.35787	.12487
		GROUP 3	-.181400	.089621	.198	-.42277	.05997
		GROUP 4	-.090400	.089621	.745	-.33177	.15097
	GROUP 2	GROUP 1	.116500	.089621	.569	-.12487	.35787
		GROUP 3	-.064900	.089621	.887	-.30627	.17647
		GROUP 4	.026100	.089621	.991	-.21527	.26747
	GROUP 3	GROUP 1	.181400	.089621	.198	-.05997	.42277
		GROUP 2	.064900	.089621	.887	-.17647	.30627
		GROUP 4	.091000	.089621	.742	-.15037	.33237
	GROUP 4	GROUP 1	.090400	.089621	.745	-.15097	.33177
		GROUP 2	-.026100	.089621	.991	-.26747	.21527
		GROUP 3	-.091000	.089621	.742	-.33237	.15037
Ra - 2 WEEKS OF REMINERALIZATION	GROUP 1	GROUP 2	.173200*	.057814	.024	.01749	.32891
		GROUP 3	.268900*	.057814	.000	.11319	.42461
		GROUP 4	.123600	.057814	.161	-.03211	.27931
	GROUP 2	GROUP 1	-.173200*	.057814	.024	-.32891	-.01749
		GROUP 3	.095700	.057814	.362	-.06001	.25141
		GROUP 4	-.049600	.057814	.826	-.20531	.10611
	GROUP 3	GROUP 1	-.268900*	.057814	.000	-.42461	-.11319
		GROUP 2	-.095700	.057814	.362	-.25141	.06001
		GROUP 4	-.145300	.057814	.075	-.30101	.01041
	GROUP 4	GROUP 1	-.123600	.057814	.161	-.27931	.03211
		GROUP 2	.049600	.057814	.826	-.10611	.20531
		GROUP 3	.145300	.057814	.075	-.01041	.30101

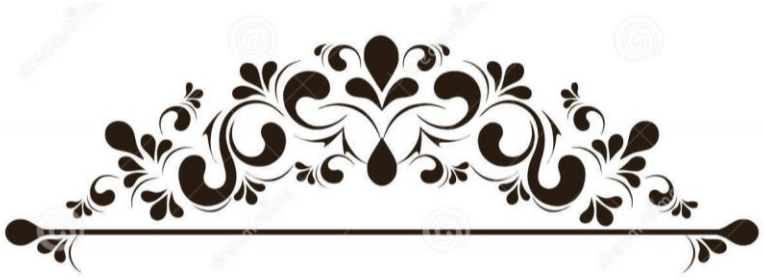
Ra - 4 WEEKS OF REMINERALIZATION	GROUP 1	GROUP 2	.181900*	.055975	.013	.03115	.33265
		GROUP 3	.337200*	.055975	.000	.18645	.48795
		GROUP 4	.217000*	.055975	.002	.06625	.36775
	GROUP 2	GROUP 1	-.181900*	.055975	.013	-.33265	-.03115
		GROUP 3	.155300*	.055975	.041	.00455	.30605
		GROUP 4	.035100	.055975	.923	-.11565	.18585
	GROUP 3	GROUP 1	-.337200*	.055975	.000	-.48795	-.18645
		GROUP 2	-.155300*	.055975	.041	-.30605	-.00455
		GROUP 4	-.120200	.055975	.158	-.27095	.03055
	GROUP 4	GROUP 1	-.217000*	.055975	.002	-.36775	-.06625
		GROUP 2	-.035100	.055975	.923	-.18585	.11565
		GROUP 3	.120200	.055975	.158	-.03055	.27095
Ra - FINAL ACID CHALLENGE	GROUP 1	GROUP 2	.110000	.066175	.358	-.06822	.28822
		GROUP 3	.150800	.066175	.122	-.02742	.32902
		GROUP 4	.546500*	.066175	.000	.36828	.72472
	GROUP 2	GROUP 1	-.110000	.066175	.358	-.28822	.06822
		GROUP 3	.040800	.066175	.926	-.13742	.21902
		GROUP 4	.436500*	.066175	.000	.25828	.61472
	GROUP 3	GROUP 1	-.150800	.066175	.122	-.32902	.02742
		GROUP 2	-.040800	.066175	.926	-.21902	.13742
		GROUP 4	.395700*	.066175	.000	.21748	.57392
	GROUP 4	GROUP 1	-.546500*	.066175	.000	-.72472	-.36828
		GROUP 2	-.436500*	.066175	.000	-.61472	-.25828
		GROUP 3	-.395700*	.066175	.000	-.57392	-.21748

GRAPH 2: SURFACE ROUGHNESS

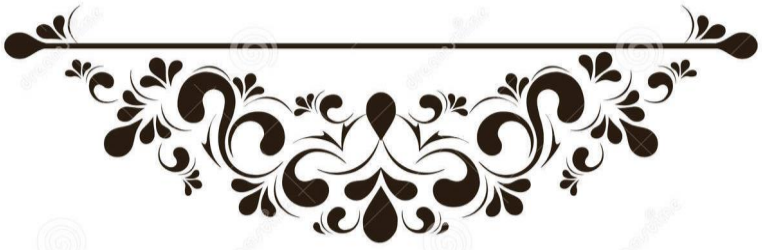


SEM MORPHOLOGICAL CHARACTERS

Sound enamel has homogeneous and a definitive smooth appearance. However, demineralized enamel showed a marked increase in the porosity with fish-scale pattern as depicted in SEM pictures (Fig 12). The test groups showed increased density of crystals after 2 weeks of remineralization. While after 4 weeks remineralization, enamel surfaces revealed a newer coating that progressively filled the pits and scratches where the prismatic enamel structures became hidden by the mineral deposition. All the specimens showed evidence of thickening of their inter-rod substance early by 2 week of remineralization however, extended remineralization period enhanced the growth of enamel crystals to cover over fish scale appearance of demineralized surface. In Group 2, after 4 weeks of remineralization, some of the enamel crystals were even fused together and were arranged homogenously with no obvious inter-crystalline spaces on their surface. After exposure to acid challenge, the experimental groups (Group 4, Group 3 and Group 2) showed more resistance to dissolution as compared to the control group (Group 1). The treated surfaces retained calcified deposits but with the presence of some potholes on their enamel surface.



DISCUSSION



DISCUSSION

Early enamel caries which are so called as the White Spot Lesions (WSL) or Incipient Lesions can histologically be described as a subsurface carious lesion of the enamel. Prominent feature being a subsurface demineralized zone with intact and also unscathed enamel surface. Even though the surface is intact, the mineral content is deficient, when opposed to sound enamel. During demineralization process, Ca2+, OH−, PO4 2−, F−, CO3−, Na+ and Mg2+ get displaced from the enamel surface to the exterior. More the acidic environment, greater is the outward flow of the ions. However, the mineral content of the surface is higher than the body of the lesion.[49]

The present study was carried out to compare the remineralizing efficacy of four regimens on artificial caries like enamel lesions, in terms of change in surface micro hardness, ultrastructure and also surface roughness. The specimens were then stored in the demineralizing solution for period of 5 days until a uniform white spot lesion were created on the surface. And this resulted in a subsurface demineralization with an intact surface replicating an early enamel lesion similar protocol was followed by **Gangrade** et al [45] unlike other studies (**Krishnan et al,[26] Elkas et al[21]**) where the specimens were polished flat using grit silicon carbide paper; it's done in order to remove the prismless enamel layer that arises at the end of amelogenesis. Although this layer is not so frequently found on the surface of permanent teeth compared to their predecessors, it is known that the prism-free enamel is gradually worn off during mastication but it is retained in protected areas.

Flat and polished specimens were used in those studies in an attempt to remove natural variations on surface enamel between teeth and between different tooth sites and types, which may result in different responses to acid dissolution. But the areas that are

subjected for demineralization did not represent an ideal enamel surface, as removal of the outer surface layer made the enamel more susceptible for demineralization process.[21,26]

Micro-hardness testing is considered to be relatively simple and reasonably reliable method for the provision of indirect information about the mineral content changes of hard dental tissues. Vickers surface micro-hardness technique has been utilized as an indirect mineral content assessment method in several laboratory models and simulating the effect of application of various commercial products on in vitro. [51]

Surface roughness assessment is an important aspect as it may not only affect aesthetic properties but also reflects the bacterial adhesion along with plaque formation potentials in the oral environment and tends to fulfil the need for quantitative characterization of surface topography with contact profilometer. [50] SEM is a complementary tool, which helps to elucidate the surface ultra-morphological changes induced by various remineralizing agents. [52]

The initial mean VHN values for the four groups ranged from 262.077 to 286.473 and Surface Roughness values ranged from .49140 to .52170 for human teeth enamel. After demineralization, the VHN values apparently decreased (172.324 to 189.467), surface roughness significantly increased (1.473 to 1.655), and enamel surface micro-porosities increased as depicted in the SEM pictures. The net remineralization produced by saliva is small and is a slower process, with a tendency for the mineral gain to be in the surface layer of the lesion due to the low ion concentration gradient from saliva into the lesion.[53]

Thus, the control group showed the least values of remineralization based on the limited availability of calcium and phosphorous ions compared to the remineralizing effect of other agents applied and this result was in accordance with the study done by

Tahmasbi et al.[36] In addition, the formula of the artificial saliva (**Torres et al.)**[51] used in the present study did not contain fluoride component which could explain the limited ability for remineralization efficacy of the control group.

Despite of different forms of calcium and phosphorous compounds incorporated in all these products, all remineralizing agents examined showed higher remineralizing potential compared to their control in terms of increase in VHN and reduction in Ra values with reduction in the surface micro-porosities as presented in SEM photos.

CPP-ACPF (casein phosphopeptide- amorphous calcium phosphate with 900ppm of Fluoride) (Tooth Mousse Plus – GC India) technology deliver ACP and fluoride compounds to the tooth structure that readily solubilize to calcium, phosphate and fluoride ions when comes in contact with saliva, creating a supersaturated state of calcium, phosphate and fluoride around the tooth enamel.[13] In CPP-ACPF technology, ACP is stabilized by CPP which is casein-derived peptides. CPP contains the amino acid cluster sequence –Ser(P)-Ser(P)- Ser(P)-Glu-Glu– and have been reported to bind amorphous calcium phosphate, forming minute clusters of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). This helps to prevent these clusters from reaching the critical size for their precipitation, thereby, stabilizing calcium phosphates in solution in close proximity of the tooth making it available whenever needed. These nanocomplexes act as calcium and phosphate reservoirs when incorporated into the dental plaque and on the tooth structure.[12]

In this technology two phase delivery system is used to keep the calcium and phosphorus components from reacting with each other before their use. The source of calcium and phosphates are composed of two salts, calcium sulfate and dipotassium phosphate. When these two salts are mixed, they rapidly form CPP-ACPF that precipitate

on tooth surface. The precipitated component readily dissolves into saliva to be available for remineralization of the tooth surface.[17]

Remin Pro® (VOCO) is a water-based cream, which contains hydroxyapatite, fluoride and xylitol (artificial sweetner). Hydroxyapatite fills the superficial enamel lesions and the tiniest irregularities that arise from demineralization. 1,450ppm of Fluoride gets converted to fluorapatite when it comes in contact with saliva; thus, strengthens the tooth and renders it to more resistant to acid attacks. Xylitol reduces the harmful effects of bacteria and their metabolic product like lactic acid. This product has been assumed to be suitable for management of dentinal hypersensitivity, prevention of enamel demineralization and promoting remineralization of enamel subsurface lesions.[54]

CaSP (Calcium Sucrose Phosphate) (Enafix – Group Pharmaceuticals) is a combination of calcium salts of sucrose and phosphate esters, mixed with inorganic calcium. It readily breaks down and releases calcium ions, phosphate ions, and sucrose phosphate ions into the saliva. It is composed of 10%–12% calcium (wt%) and 8%–10% phosphorous (wt%). Calcium and phosphate ions in aqueous media forms an insoluble precipitates. CaSP forms an aqueous solutions consisting of high concentration of calcium and phosphate without occurrence of precipitation. It acts as an ideal carrier for the calcium and phosphate in water. Enafix acts by adsorption of sucrose phosphate ion rapidly on the enamel surface, thereby reducing the rate of acid dissolution of hydroxyapatite and quick remineralization by calcium and phosphate ion by the common ion effect.[23]

In this study the artificial saliva was prepared according to **Torres** et al [51] formula and consisted of 22.1mM hydrogen carbonate, 16.1mM potassium, 14.5mM sodium, 2.6 mM hydrogen phosphate, 0.8mMboric acid, 0.7mMcalcium, 0.2mM thiocyanate, and 10.2mM magnesium. The pH was adjusted between 7.4 and 7.8. [51] The

use of artificial saliva in the present study cannot model remineralization and demineralization process in intraoral environment which is related to various biological factors, such as salivary pellicle and biofilm. Natural saliva offers a greater protection than artificial saliva due to its ability to form salivary pellicle that restricts the acid diffusion and transport of ions in and out of the enamel surface.

During the process of enamel remineralization the crystal voids repair, the degree of remineralization and the repaired crystalline phase formed will depend on the concentration of bioavailable fluoride, calcium and phosphate ions. However several studies reported that the bioavailability of active fluoride ions is the critical factor for promoting remineralization and inhibition of demineralization in calcium- and phosphate rich circumstance.[55]

Taking the previous element into consideration the CaSP group has the increased resistance to final acid challenge when compared to all the other groups. However fluoride catalysis the diffusion of calcium and phosphate into the tooth surface, which in turn remineralize the crystalline structures in dental cavities leading to the formation of the fluorapatite crystals. Taking all the previous elements in account might help to interpret the results that were obtained. It was found that CPP-ACPF group showed the highest remineralizing potential when compared to all other groups, in terms of highest VHN, lowest Ra values, and reduction in the surface micro-porosities as depicted in SEM pictures were also appreciable.

Reynolds et al in their study also identified the role of casein phosphopeptides (CPP) in the stabilization and localization of amorphous calcium phosphate (ACP) at the level of tooth sub-surfaces. In their in vitro study, they showed that CPP-stabilized calcium phosphate solutions were shown to remineralize subsurface lesions in human third-molar enamel. Although most of the remineralizing solutions were supersaturated

with respective amorphous and crystalline calcium phosphate phases, the solutions were stabilized by the CPP such that spontaneous precipitation of calcium phosphate doesn't occur. And the study concluded that the remineralizing capacity was greater for the solutions with the higher levels of CPP-stabilized free calcium and phosphate ions and so remineralization was not significantly correlated with either the CPP-bound ACP or the degrees of saturation for hydroxyapatite, octacalcium phosphate, or ACP.[12]

Sharma et al [43] evaluated the remineralization potential of two commercially available pastes, containing CPP–ACP and HAp, on the demineralized enamel surface. Although statistically not significant, in this study, ReminPro yielded better results with regard to microhardness than GC Tooth Mousse. The difference between the results of the two materials was substantially more evident after 30 days, indicating that ReminPro had better long-term effect as compared to GC Tooth Mousse. ReminPro consists of additional components of fluoride and xylitol, which may aid in increasing the remineralization potential of the paste but the CPP-ACP cream used in their study does not contain fluoride unlike our study where we compared the remineralizing efficacy of CPP – ACPF (GC Tooth Mousse Plus) with 900 ppm of fluoride is found superior to the reminiralizing efficacy of HAp (ReminPro) with 1,450ppm of fluoride. [43]

Elkassas D et al conducted a study to assess enamel remineralization of ClinproTM white varnish, Tooth Mousse Plus and VanishTMXT and evaluated their remineralizing efficiency at baseline, after demineralization, after 2 and 4 weeks remineralization and after final acid challenge. And concluded that ClinproTM varnish presented the highest remineralization tendency with greatest resistance for the final acid challenge. [21]

Jayrajan et al evaluated the efficacy of CPP-ACP and CPP-ACPF on the surface enamel remineralization using SEM and DIAGNOdent and they concluded that a

combination of CPP–ACP and fluoride would result in co-localization of calcium and phosphate ions with fluoride ions at the enamel surface, presumably as CPP–ACFP nanocomplexes and so CPP-ACPF could be included in the routine hygiene and maintenance for reversing or arresting white spot lesions.[24]

Lata et al compared the remineralization potential of NaF and CPP-ACP; they reported that the remineralization potential of NaF was higher than that of CPP-ACPF. Also, the remineralization potential of fluoride alone was higher than that of CPP-ACPF that where evaluated with SMH values.[49] Such a difference in the results might be due to the different demineralising solution for the creating artificial caries like lesion. In their study demineralizing solution were prepared with Hanning et al formula which consisted of CaCl2 (2.2 mM), NaH2PO4 (2.2 mM), lactic acid (0.05 M), fluoride (0.2 ppm) with a pH of 7.1.[56] While in our study the demineralising solution was made in correlation with Pulido et al The proposal suggested by **Pulido et al 2008** [13] has been followed for creating artificial caries like lesions, with the solution containing 2.2 mM CaCl2_2H2O, 2.2mM KH2PO4, 0.05M acetic acid and pH adjusted to 4.4 with 10 MKOH.

Our study was in accordance to the study done by **Gade et al** who compared the remineralizing efficacy of CaSP (Enafix) with CPP-ACPF (Tooth Mousse Plus) and **Gade et al** suggested that CaSP compared to CPP-ACPF has better properties in withstanding final acid challenge and so in their study they stated that Enafix being a cost effective material as compared to GC Tooth mousse plus and moreover it can be used as an alternative regime for the purpose of better remineralization specially in Indian scenario.[48]

Gangrade et al evaluated the remineralizing potential among SnF, CPP-ACPF and CaSP and concluded that the remineralizing efficacy of SnF is found superior to the

other two remineralizing agents.[45] In their study, CPP-ACPF got the least mean microhardness of 200.81 **MPa** to the maximum while in our study the SMH value of remineralization of CPP-ACPF was about 296 .510 MPa to the maximum and was found superior in remineralization while compared to the other products. This contradictory result might have occurred due to shorter duration (7 days) of remineralization in their study.

Sharma et al conducted a study to analyze the caries inhibitory potential of CPP ACPF(GC Tooth Mousse Plus) and CaSP (Enafix) and concluded that CPP-ACPF application was observed as the most effective surface treatment modality when compared to CaSP. [57] This contradictory result could be attributed because the samples were not acid challenged for activation of the delivery molecules prior to remineralization cycle and they were challenged to demineralization cycle only once, unlike in our study the samples were acid challenged before as well as after the remineralization cycle.

Another study done by **Kakkar** et al that lies in accordance to our study where they conducted a repeated demineralization and remineralization cycle to compare the remineralizing ability and their efficiency to withstand the acid challenge demineralization with etched enamel surface during bonding among the various remineralization agents like artificial saliva, Fluor-Protector group, GC Tooth Mousse Plus group, Enafix group and Amflor group. They found that GC Tooth Mousse Plus group was found the best among different medicaments, however it was not statistically significant, while Enafix is a complex mixture of calcium sucrose phosphate along with inorganic ACP. During brushing, calcium sucrose phosphate (anticay) in Enafix quickly breaks down and releases calcium, phosphate, and sucrose phosphate ions into saliva. [58]

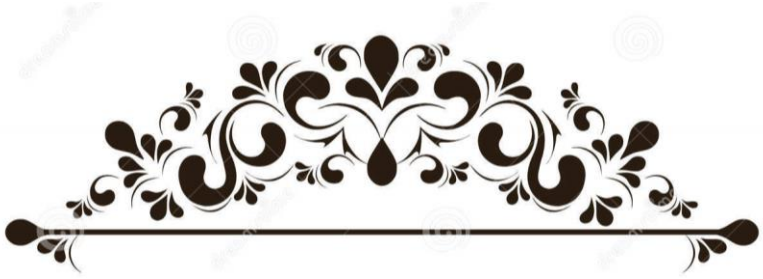
Calcium and phosphate ions rapidly gets adsorbed onto the enamel and decreases the rate of enamel solubility under acidic conditions and at neutral pH, and increase the rate of remineralization. The added factor is that sucrose phosphate anions gets adsorbed onto the surface of tooth and reduced the rate of acid dissolution. However, the short comings of this inorganic fluoride in comparison to organic fluoride present in Enafix are that which basically forms a thick layer of calcium fluoride by reaction with hydroxyapatite of the surface enamel. And due to this thick layer of calcium fluoride formed, there is lower bioavailability of fluoride ions. But however they concluded that although Amflor and Enafix were not found statistically significant from the controlgroup, they also prevent the enamel demineralization and moreover they are also cost-effective.[58]

Sandeep et al in their study used the remineralizing agents such as Fluoride enriched Casein Phospho-peptide- Amorphous Calcium Phosphate [CPP - ACPF] (GC Tooth Mousse Plus), Beta Tri- calcium Phosphate[β-TCP] (3M ESPE ClinPro) and Hydroxyapatite [HA] (Reminpro VOCO) based cream on bleached enamel surface was evaluated in this study, using Vicker's hardness test. Within the limitation of their in vitro study, it can be concluded that bleaching results in statistically significant loss of minerals from enamel surface when compared to that of the intact sound enamel. All the remineralizing agents tested were found to be effective in remineralizing the bleached enamel surface in the following order: HA, CPP-ACPF and then β-TCP. Among the remineralizing agents tested in the study, Hydroxyapatite based cream was found to be marginally more effective than the CPP-ACPF and β-TCP based products however it was not statistically significant and in contradictory to our study. In their study the CPP-ACPF treated group showed the second highest microhardness recovery, which was

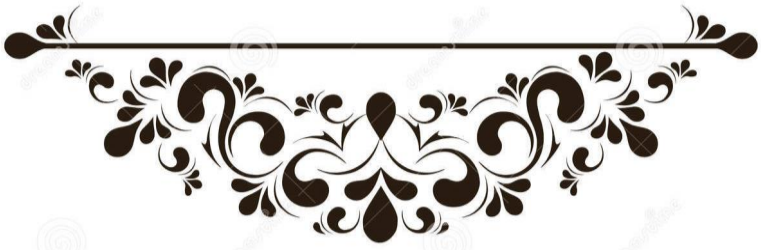
statistically significant when compared with the control Group but not with Hydroxyapatite Group and Grp 3 (β-TCP). [29]

This is not in agreement with this study and it can be due to the fact that the peptides in the CPP–ACP might diffuse into the inter-crystallite spaces, (as the size of these peptide complex are small [~2 nm]) created by bleaching. It localises and increases the bioavailability of calcium and phosphate ions and it may enhance the potential for remineralization and deposition of minerals around the "freshly cleaned" enamel crystallites, leading to an increase in hardness thus regaining the prebleaching enamel physical properties. Incorporation of fluoride into CPP-ACP as CPP-ACPF nanocomplex co-localizes fluoride ions along with calcium and phosphate, maintaining a state of super saturation on the tooth surface; hence, resulting in higher levels of remineralisation and fluoride incorporation into the active mineral phase.[29]

Another study by **Tahmasbi** et al whose results are in significant with our results, who evaluated the remineralizing efficacy of Remin Pro, CPP-ACP-F and NaF in treatment of WSLs (White Spot Lesions) using Surface MicroHardness values. And they concluded that MI Paste Plus and to a lesser degree 0.05% NaF are effective for treatment of WSLs. Although Remin Pro had an efficacy superior to that of artificial saliva, its remineralizing effect seems to be inconsistent.[36]



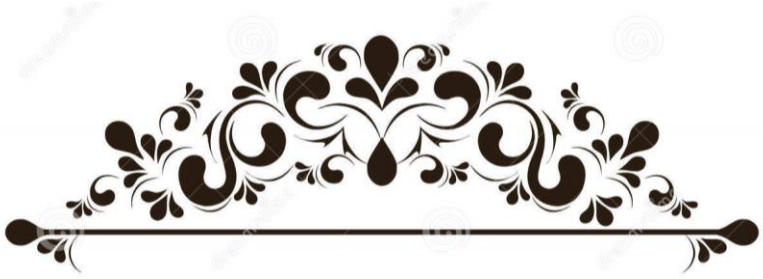
CONCLUSION



CONCLUSION

Within the limitations of this particular in-vitro study, the following conclusions are so being derived:

1. Calcium phosphate based remineralizing agents provides superior remineralization effects with increased surface hardness and reduced surface roughness and greater resistance to acid softening and all the treated surfaces retained calcified deposits when compared to artificial saliva.
2. Extended period of time had helped to attain more benefits of remineralization regimens.
3. Caesin phosphopeptide amorphous calcium phosphate containing 900ppm of fluoride has better remininerализation when compared to other groups.
4. Calcium Sucrose Phosphate has the highest resisitant to acid attack when compared to other groups.
5. Enafix being a cost effective material when compared to GC Tooth Mousse Plus, can be used as an alternative for better remineralization especially for the Indian population.



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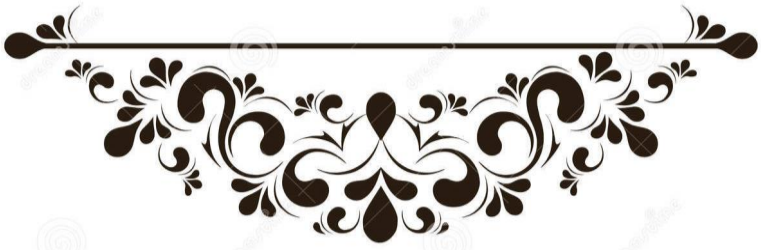

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ANNEXURES

SURFACE HARDNESS VALUES

ARTIFICIAL SALIVA

BASELINE VALUES

SAMPLE NO:	VALUES
1	294.118642
2	284.554762
3	287.267312
4	301.116782
5	270.455299
6	287.942657
7	286.116854
8	302.490701
9	278.465942
10	286.112657

INTIAL DEMINERALIZATION

SAMPLE NO:	**VALUES**
1	185.6974
2	188.6428
3	194.0121
4	187.4298
5	189.5267
6	189.1156
7	192.6387
8	184.9219
9	186.4562
10	185.6595

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	VALUES
1	170.831962
2	168.995544
3	158.942315
4	157.866038
5	165.118268
6	164.349000
7	163.895623
8	168.324985
9	156.834294
10	164.116852

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	VALUES
1	182.634525
2	180.314828
3	185.856149
4	183.998664
5	184.861252
6	184.856842
7	182.996584
8	187.995652
9	189.405172
10	186.112647

FINAL ACID CHALLENGE

SAMPLE NO:	VALUES
1	168.975134
2	163.189093
3	170.384628
4	169.994351
5	170.664853
6	171.119456
7	176.562438
8	177.580907
9	175.664523
10	171.008562

REMINPRO

BASELINE VALUES

SAMPLE NO:	VALUES
1	268.994526
2	267.669810
3	285.654284
4	277.116482
5	276.915321
6	281.365214
7	286.154190
8	269.554852
9	276.654852
10	278.334913

INTIAL DEMINERALIZATION

SAMPLE NO:	VALUES
1	176.5612
2	175.7444
3	183.8865
4	184.9942
5	185.8856
6	185.7240
7	189.3352
8	193.5542
9	189.5623
10	195.7036

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	VALUES
1	199.654253
2	198.029785
3	201.654852
4	202.856423
5	204.652355
6	204.521394
7	210.522215
8	205.645262
9	209.321862
10	203.521354

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	219.564741
2	215.528833
3	222.654255
4	221.659856
5	223.564264
6	226.432683
7	232.105167
8	223.451236
9	230.654123
10	216.842913

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	189.642519
2	174.449377
3	185.542692
4	187.264135
5	182.642535
6	183.654298
7	190.806623
8	187.523641
9	176.521468
10	182.642954

TOOTH MOUSSE PLUS

BASELINE VALUES

SAMPLE NO:	VALUES
1	258.654213
2	254.669810
3	277.116785
4	285.114632
5	275.364289
6	279.665231
7	276.224985
8	289.662459
9	275.641283
10	297.154190

INTIAL DEMINERALIZATION

SAMPLE NO:	**VALUES**
1	183.1246
2	185.6423
3	165.7444
4	183.74623
5	195.7036
6	200.6991
7	183.42394
8	192.42617
9	187.32145
10	183.62451

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	VALUES
1	198.330072
2	199.325641
3	214.136425
4	215.276000
5	215.523645
6	216.631425
7	228.654123
8	214.361758
9	232.651928
10	230.413648

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	228.321456
2	225.756800
3	244.325647
4	234.214369
5	244.170000
6	246.321458
7	245.652314
8	260.589632
9	262.583200
10	245.325647

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	173.621498
2	170.332347
3	187.107000
4	188.332419
5	185.662317
6	187.632495
7	200.621479
8	203.881653
9	198.332647
10	184.362147

ENAFIX

BASELINE VALUES

SAMPLE NO:	VALUES
1	240.135406
2	241.63215
3	259.173362
4	261.541392
5	262.077000
6	265.118631
7	262.792352
8	283.954136
9	263.115682
10	284.018594

INTIAL DEMINERALIZATION

SAMPLE NO:	**VALUES**
1	161.81321
2	160.6499
3	172.6426
4	171.5634
5	173.64289
6	172.21498
7	177.62389
8	179.652347
9	183.99815
10	179.24693

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	VALUES
1	184.684607
2	196.245624
3	201.632424
4	196.693000
5	206.267493
6	208.701393
7	201.648523
8	195.987423
9	198.352189
10	186.742982

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	215.416213
2	205.084219
3	208.641298
4	220.486953
5	215.261556
6	217.329512
7	218.659321
8	209.349163
9	225.684129
10	214.434565

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	179.324985
2	177.710875
3	193.658000
4	207.362485
5	190.658923
6	191.623547
7	193.652743
8	209.265125
9	192.556321
10	194.221475

SURFACE ROUGHNESS VALUES

ARTIFICIAL SALIVA

BASELINE VALUES

SAMPLE NO:	VALUES
1	0.56388
2	0.49563
3	0.55128
4	0.52145
5	0.51236
6	0.57432
7	0.47952
8	0.1746
9	0.49623
10	0.524136

INTIAL DEMINERALIZATION

SAMPLE NO:	VALUES
1	1.45239
2	1.28472
3	1.55246
4	1.47523
5	1.47352
6	1.29562
7	1.66308
8	1.6423
9	1.35429
10	1.47356

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.44629
2	1.32273
3	1.42613
4	1.43864
5	1.46535
6	1.44420
7	1.33542
8	1.46823
9	1.56567
10	1.55486

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.33526
2	1.46235
3	1.31474
4	1.41395
5	1.45235
6	1.41070
7	1.48623
8	1.41165
9	1.41056
10	1.50666

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	1.47536
2	1.31801
3	1.45263
4	1.35689
5	1.47235
6	1.46853
7	1.48623
8	1.62239
9	1.60523
10	1.52463

REMINPRO

BASELINE VALUES

SAMPLE NO:	VALUES
1	0.50183
2	0.53299
3	0.495315
4	0.48325
5	0.49146
6	0.50436
7	0.45265
8	0.51235
9	0.44985
10	0.46135

INTIAL DEMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.59423
2	1.48415
3	1.57462
4	1.57313
5	1.59623
6	1.47235
7	1.49653
8	1.62731
9	1.69668
10	1.68531

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.27155
2	1.21457
3	1.27365
4	1.24943
5	1.27523
6	1.29865
7	1.25965
8	1.24628
9	1.32743
10	1.30568

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.23459
2	1.13578
3	1.24892
4	1.21619
5	1.22562
6	1.15464
7	1.22649
8	1.26833
9	1.32182
10	1.30629

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	1.35456
2	1.30128
3	1.36483
4	1.36445
5	1.34892
6	1.34526
7	1.33562
8	1.41912
9	1.37456
10	1.40657

TOOTH MOUSSE PLUS

BASELINE VALUES

SAMPLE NO:	VALUES
1	0.51462
2	0.48488
3	0.49135
4	0.55423
5	0.51470
6	0.52353
7	0.51738
8	0.50436
9	0.54452
10	0.53746

INTIAL DEMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.65246
2	1.79148
3	1.52746
4	1.58235
5	1.65234
6	1.76235
7	1.66482
8	1.63535
9	1.51912
10	1.61253

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.22796
2	1.17536
3	1.16834
4	1.17535
5	1.12546
6	1.17493
7	1.12567
8	1.15326
9	1.18366
10	1.12264

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	VALUES
1	0.98562
2	1.06246
3	0.96039
4	0.994351
5	1.07356
6	1.09453
7	1.07350
8	1.15356
9	1.18043
10	1.18661

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	1.25364
2	1.22698
3	1.34895
4	1.33589
5	1.39545
6	1.31940
7	1.35356
8	1.31456
9	1.40646
10	1.41182

ENAFIX

BASELINE VALUES

SAMPLE NO:	VALUES
1	0.40267
2	0.42356
3	0.47531
4	0.50135
5	0.48526
6	0.45268
7	0.46235
8	0.46135
9	0.45862
10	0.51333

INTIAL DEMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.43554
2	1.56264
3	1.46256
4	1.63566
5	1.56483
6	1.55235
7	1.55326
8	1.56489
9	1.69307
10	1.67235

2 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.41265
2	1.43678
3	1.35436
4	1.32546
5	1.40656
6	1.32054
7	1.310543
8	1.25364
9	1.20442
10	1.29489

4 WEEKS OF REMINERALIZATION

SAMPLE NO:	**VALUES**
1	1.18135
2	1.16423
3	1.15612
4	1.19453
5	1.12468
6	1.19356
7	1.15462
8	1.19463
9	1.23568
10	1.20654

FINAL ACID CHALLENGE

SAMPLE NO:	**VALUES**
1	0.90456
2	0.82549
3	1.65364
4	0.92468
5	0.92375
6	1.02462
7	0.93465
8	1.02191
9	1.01686
10	0.954355